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THE STRUCTURE OF BIOTIN*

By Dr. VINCENT du VIGNEAUD

PROFESSOR OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE

DURING the past year my associates and I have been working on the structure of biotin and I should like to take this opportunity of presenting to you the results of this study. In 1940, our group at Cornell University Medical College, in collaboration with Dr. Paul György and Catharine S. Rose at Western Reserve, had demonstrated that biotin, the yeast-growth substance which had been isolated by Kögl, was actually identical with vitamin H.^{1, 2, 3} Vitamin H was the name which had been given by György to the fac-

tor present in liver, yeast and various foods which was capable of preventing the fatal syndrome resulting from the feeding of large amounts of raw egg white, a syndrome found to occur in all species studied. We were thus able to show that biotin was involved in animal metabolism and through this work biotin became recognized as a member of the vitamin B-complex. The full role in nutrition of this newcomer to the group of vitamins is not fully understood, yet there are indications that it may be extremely important. There are now scores of laboratories working on this compound and within the next year or two much light should be thrown on the significance of this vitamin. With the demonstration of the identity of vitamin H with biotin we undertook a study of the chemical nature of this compound and have recorded from time to time some of our chemical findings. We

* A lecture delivered before the New York Section of the American Chemical Society, on October 9, 1942.

¹ P. György, D. B. Melville, D. Burk and V. du Vigneaud, SCIENCE, 91: 243, 1940.

² V. du Vigneaud, D. B. Melville, P. György and C. S. Rose, SCIENCE, 92: 62, 1940.

³ P. György, C. S. Rose, K. Hofmann, D. B. Melville and V. du Vigneaud, SCIENCE, 92: 609, 1940.

have announced for example, that biotin is a cyclic urea derivative^{4, 5}; that it contains sulfur in thio ether linkage⁴; that through the oxidation of the compound one can obtain adipic acid;⁶ and that after the elimination of the carboxyl group of biotin, adipic acid can no longer be obtained.⁷ On the basis of these facts and on consideration of the saturated character of the compound and the empirical formula, we were led to suggest that biotin was a bi-cyclic compound and that there were 5 structures capable of explaining the data up to that time; that is, last January.⁸ We have now gone further with the study and have arrived at what we believe with considerable confidence to be the structure of biotin. I propose to confine my presentation to this actual chemical work, foregoing consideration of the biological aspects. I have earlier reviewed the historical side of the problem.⁹

In presenting these structural studies, I would like to pay tribute to the teamwork of the group participating in the work. I would like to acknowledge in particular the splendid contributions which Dr. Melville and Dr. Hofmann have made in this degradation work. Drs. Brown, Kilmer and Armstrong of our group have also made important contributions to the problem in connection with the synthesis of ring sulfur compounds which have helped us to understand certain aspects of biotin chemistry.¹⁰ I wish to acknowledge the cooperation of Mr. Frohring and the Research Staff of the S.M.A. Corporation and Dr. Major and the Research Staff of the Merck Research laboratories for supplies of crystalline material. I would also like to acknowledge the collaboration in a certain phase of the work on desthiobiotin of a group from the Merck laboratories; namely, Drs. Folkers, Wolf, Keresztesy, Harris and Mzingo. I shall mention others of the group in the course of the discussion who have likewise made valuable contributions to the work. Finally I would like to acknowledge the benefit of many fruitful discussions with Professor Hans Clarke, who followed step by step the course of these studies with such great interest.

By chromatographic procedures which we have already described^{11, 12} we were able to isolate biotin

⁴ K. Hofmann, D. B. Melville and V. du Vigneaud, *Jour. Biol. Chem.*, 141: 207, 1941.

⁵ D. B. Melville, K. Hofmann and V. du Vigneaud, *SCIENCE*, 94: 308, 1941.

⁶ K. Hofmann, D. B. Melville and V. du Vigneaud, *Jour. Am. Chem. Soc.*, 63: 3237, 1941.

⁷ K. Hofmann, D. B. Melville and V. du Vigneaud, *Jour. Biol. Chem.*, 144: 513, 1942.

⁸ V. du Vigneaud, K. Hofmann and D. B. Melville, *Jour. Am. Chem. Soc.*, 64: 188, 1942.

⁹ V. du Vigneaud in Evans, "The Biological Action of the Vitamins," University of Chicago Press, 1942.

¹⁰ G. W. Kilmer, G. B. Brown, M. D. Armstrong and V. du Vigneaud, *Jour. Biol. Chem.*, 145: 495, 1942.

¹¹ V. du Vigneaud, K. Hofmann and D. B. Melville, *Jour. Biol. Chem.*, 140: 643, 1941.

from liver extracts and from milk concentrates. The compound was isolated as the methyl ester, which by repeated crystallizations from a mixture of methanol and ether was obtained in long, thin, plate-like needles. The ester melted sharply on the hot-stage at 166–167°. This melting point was considerably higher than that reported by Kögl and Tönnis.¹³ Subsequently Kögl has reported that his material was impure and he has now reported a melting point which is substantially in agreement with ours.¹⁴ A chloroform solution of the ester showed an optical rotation of +57°.

Expressed in terms of vitamin H units the various preparations of purified product that we prepared all consistently yielded, by the yeast-growth method, the high value of 27,000 (± 10 per cent.) vitamin H units per mg. (The vitamin H unit is the amount necessary per day for 30 days to cure egg white deficiency symptoms.) Half-maximum growth of the yeast culture was obtained at a concentration as little as 1 part in 1×10^{10} , which indicates the tremendous activity of this material. Direct vitamin H assays of the crystals by Dr. György, carried out with rats by the curative method, were in agreement with this high potency. This means that approximately 0.04 γ per day suffices to prevent the fatal syndrome resulting from the egg-white diet employed in the feeding of the rats, truly an amazing potency.

The analytical values we obtained from the pure crystalline compound agreed most closely with the empirical formula of $C_{11}H_{18}O_3N_2S$, which agrees with that given by Kögl. The free biotin was readily obtained by saponification of the ester with cold alkali.¹⁵ Upon acidification of the saponification mixture with HCl, free biotin separated in long, thin needles. The analytical figures pointed to the composition $C_{10}H_{16}O_3N_2S$, which is in good agreement with the composition of the ester. An alkaline solution of the biotin showed an optical rotation of +92°. The titration curve run by Dr. Rachele of our laboratory, who likewise carried out all the micro analyses, resembled the titration curve of a simple monocarboxylic acid. The neutral equivalent of 244 obtained from the curve agreed with that expected for a monocarboxylic acid of the empirical formula given. In the yeast-growth assay the free biotin appears to have the same potency per mole as the ester. For some micro-organisms it is necessary, however, to have biotin in the free form and not as the ester.

With the crystalline material available it was pos-

¹² D. B. Melville, K. Hofmann, E. Hague and V. du Vigneaud, *Jour. Biol. Chem.*, 142: 615, 1942.

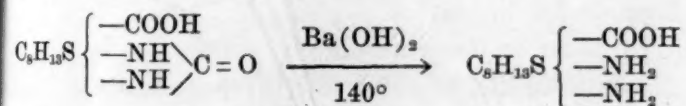
¹³ F. Kögl and B. Tönnis, *Z. Physiol. Chem.*, 242: 43, 1936.

¹⁴ F. Kögl and L. Pons, *Z. Physiol. Chem.*, 269: 61, 1941.

¹⁵ V. du Vigneaud, K. Hofmann, D. B. Melville and J. R. Rachele, *Jour. Biol. Chem.*, 140: 763, 1941.

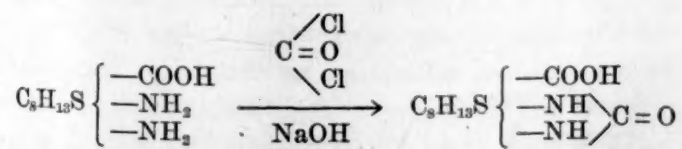
sible to obtain preliminary information on the stability and behavior of the compound towards various reagents by inactivation and reactivation experiments using small amounts of material.¹⁶

In actually tackling the characterization of the functional groups by direct chemical means we first directed our attention towards the nitrogen and oxygen. Two of the three oxygen atoms, of course, had been accounted for by the carboxyl group. Possibility after possibility of how the remaining oxygen and two nitrogen atoms were arranged was eliminated. There is no point, however, in going into the many negative experiments in this direction. It became very puzzling as to what the nature of the nitrogen might be. However, the break came when a cleavage product was obtained after treatment of the biotin with strong barium hydroxide for 20 hours at 140°. This brought about the formation of a new diamino acid which could be isolated in excellent yield. The analysis of the free compound led to the empirical formula $C_9H_{15}O_2N_2S$. It was clear that the split product had lost one carbon and one oxygen and taken up two hydrogens. No loss of anything else occurred. The most logical interpretation we could place on this was the cleavage of a cyclic urea derivative. The hydrolytic cleavage of biotin could therefore be expressed by the following equation.



You will note that the urea structure and the carboxyl group accounted for all the oxygen and the nitrogen, leaving the sulfur to be accounted for. Again many possibilities were eliminated and we suspected that the sulfur was present as a thio ether. A second break came when a crystalline sulfone ($C_{10}H_{16}O_5N_2S$) was obtained by the action of H_2O_2 , which led to our recognition that the sulfur was present as a thio ether.⁴

It is obvious that if biotin were a urea derivative and if the barium hydroxide treatment yielded a diaminocarboxylic acid then we should be able to resynthesize biotin from the diaminocarboxylic acid by closing the ring again through urea formation. This we were able to accomplish by treatment of the diaminocarboxylic acid with phosgene⁵ as shown in this equation.



By this reaction biotin of the same melting point, crystalline form and optical rotation was obtained in

98 per cent. yield. A mixed melting point of the resynthesized biotin with the isolated natural biotin showed no depression. The resynthesized biotin had the same biological activity as the naturally occurring biotin. This evidence proved beyond a shadow of doubt the cyclic urea structure of biotin.

By taking into account the absence of the ethylenic linkage as well as the nature of the functional groups and the ratio of hydrogen to carbon, we were able to arrive at the conclusion that biotin must contain a bicyclic ring system. In two papers of Kögl and co-workers,^{14, 17} identical conclusions were arrived at independently with regard to the nature of the functional groups. In addition they claimed to have obtained evidence that the sulfur is present in a ring. They claimed that they were able to cleave a carbon-sulfur bond and the urea ring of biotin sulfone at the same time and still found the 9 carbons and 2 nitrogens with the sulfur. As we have shown¹⁸ this claim was based on an erroneous deduction so that the Kögl data did not afford evidence for a sulfur-containing ring. Both their evidence and ours, independently arrived at, simply showed the nature of the functional groups.

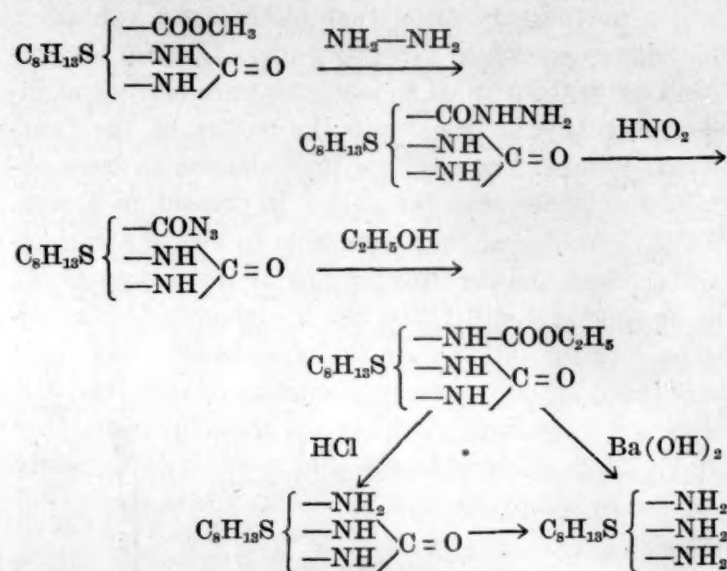
The next question, of course, was how the functional groups were arranged. As a step in this direction we subjected the diaminocarboxylic acid to oxidative degradation to see if we could pick up some characteristic split product. Fortunately we were able to obtain a split product containing 6 carbons in a chain, representing a substantial part of the 9 carbons of the diaminocarboxylic acid.⁶ The oxidative degradation was first carried out with alkaline permanganate and later with nitric acid. Out of the mixture of degradation products it was possible to isolate in good yield adipic acid, the 6-carbon dicarboxylic acid. The isolation of the same compound under both these alkaline and acid oxidizing conditions minimized to a great extent the possibility of a rearrangement to an intermediate which could have yielded adipic acid. Thus the consistent formation of adipic acid as an oxidation product of biotin could be interpreted in one of two possible ways. Either biotin contains an aliphatic side chain which is capable of yielding adipic acid; or else the adipic acid has its origin in a cyclic structure which is cleaved by the oxidation. In the first case one of the carboxyl groups of the adipic acid must be the carboxyl group originally present in biotin, and it should therefore be possible, by the oxidation of a derivative of the diaminocarboxylic acid in which the carboxyl group has been eliminated, to decide between the two alternatives. After several

¹⁷ F. Kögl and T. J. de Man, *Z. Physiol. Chem.*, 269: 81, 1941.

¹⁸ D. B. Melville, K. Hofmann and V. du Vigneaud, *Jour. Biol. Chem.*, 145: 101, 1942.

¹⁶ G. B. Brown and V. du Vigneaud, *Jour. Biol. Chem.*, 141: 85, 1941.

attempts by other methods the objective was achieved by a Curtius degradation.⁷ In this way the carboxyl group was replaced by an amino group. Biotin methyl ester was converted to the hydrazide, from which the azide was obtained by treatment with nitrous acid. The azide was transformed into the corresponding ethyl urethane. The hydrolysis of the urethane was performed in two ways as indicated in these equations.



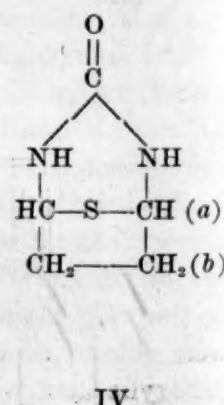
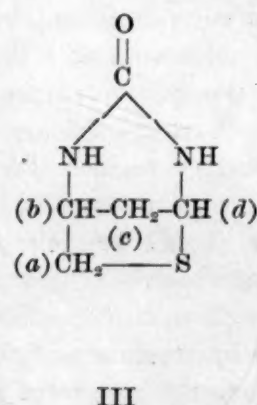
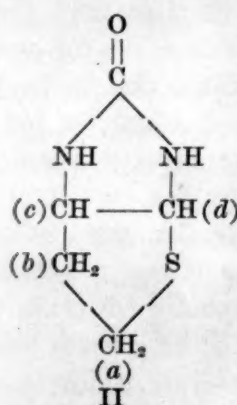
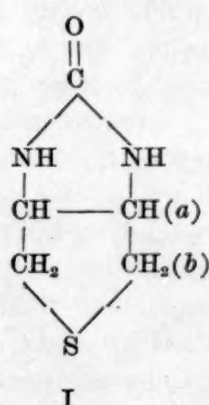
The triamine was subjected to the same oxidation procedures which we employed for the oxidation of the diaminocarboxylic acid. After preliminary experiments, 50 mg of the triamine sulfate were oxidized with potassium permanganate under the same conditions employed in the oxidation of the diaminocarboxylic acid. No trace of adipic acid could be detected in the ether-soluble oxidation products, although the amount of adipic acid which might have been formed from the relatively large amount of triamine used

giving rise to adipic acid upon oxidation is not present in biotin as a cyclic structure, but indicates the presence of an aliphatic acid side chain in biotin which is capable of yielding adipic acid on oxidation.

With all the foregoing data we were in position to list the possible structures which would fit these data. The most logical interpretation of the adipic acid data as a whole was that biotin contained a normal valeric acid side chain. The adipic acid would arise then from this side chain plus one carbon in the ring which was so linked that on oxidation it could give rise to a carboxyl group. With this deduction along with the other chemical data we had we could write, on the basis of 5 or 6 membered rings being present, only the structures indicated by formulas I, II, III and IV, with a valeric acid side chain at the positions indicated.⁸

You will note, however, that formulas II, III and IV have sulfur and nitrogen attached to the same carbon. We felt that the remarkable stability of the diaminocarboxylic acid towards strong hydrolytic agents rendered very unlikely structures where nitrogen and sulfur were attached to the same carbon. Such compounds described in the literature are unstable to strong alkali. As we stated in the preliminary note, formula I with either the side chain in position (a) or position (b) was the most likely formula for biotin.

In order to keep absolutely within the bounds of our data we had to grant another possibility although it seemed to us less likely. It was theoretically possible that the adipic acid might arise from the decarboxylation of a malonic or α -substituted β -keto acid arising during the oxidation, in which case a butyric rather than a valeric acid side chain might be present.



Side Chain = $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{COOH}$

would have made its isolation and identification comparatively easy. The absence of adipic acid in isolable amounts among the oxidation products of the triamine therefore afforded substantial evidence that one of the carboxyl groups of the adipic acid formed by oxidation of the diaminocarboxylic acid is identical with the original carboxyl group of biotin. This means in effect that the 6-carbon, straight chain moiety

On the basis of such a construction we could arrive at 3 additional structures, as shown in formulas V, VI and VII.

At this stage we therefore had before us 5 structures which we felt were the only ones which could possibly explain the chemical evidence which we had so far adduced. As we pointed out⁸ all the formulas with the exception of formula Ia involved, in the

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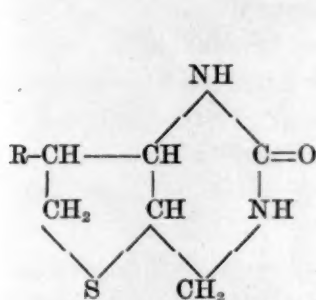
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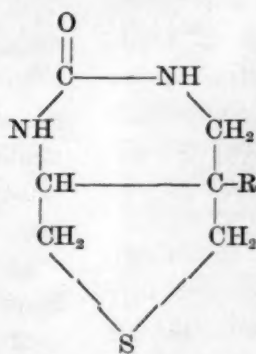
formation of adipic acid, the oxidative cleavage of a carbon-sulfur bond. As we stated it, formulas Ib, V, VI and VII involved the assumption that the carbon atom attached to the sulfur and proximal to the side chain would be oxidized to a carboxyl group. We pointed out that if this assumption were invalid then only structure Ia remained. We did not rule out this assumption. In other words we considered these formulas involving the splitting of the carbon-sulfur bond as entirely possible and our further work was based on the allowance of that assumption. This left us then at this stage with 5 possible structures for biotin, with preference for the fused 5-membered rings with a valeric acid side chain. We had hoped that x-ray data might aid us in ruling out some of the structures. Dr. Fankuchen was kind enough to make

in pale yellow needles. The analytical values for the compound agreed somewhat more closely with the composition of the quinoxaline rather than the dihydroquinoxaline derivative. The red color which was obtainable on treatment of the condensation product with sulfuric acid was in favor of this.

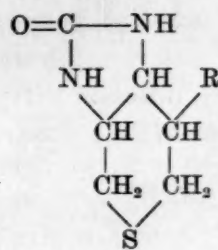
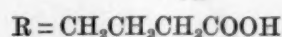
The formation of the derivative with phenanthrenequinone indicated strongly if not proved that the diaminocarboxylic acid is a 1,2 diamine and that therefore biotin possesses a 5-membered urea ring. This is in contradiction to the suggestion of Kögl and Pons¹⁴ that biotin is a 6-membered cyclic urea derivative. The evidence on which they based this suggestion was simply the comparative stability of 5- and 6-membered cyclic urea derivatives toward hydrolysis. The demonstration that the diamino-



V



VI



VII

such an analysis of biotin, but felt that the x-ray data did not warrant a decision on which structure was the more likely in the presentation of his x-ray data.¹⁹ Since our determination of the structure by chemical means he has obtained evidence in favor of it from a study of the x-ray pattern of biotin sulfone.

In the chemical attack it is obvious that an important step would be the establishment of whether the urea ring was 5- or 6-membered, or to put it another way, whether the diaminocarboxylic acid derivable from biotin was a 1,2 or 1,3 diamine. The ring closure with phosgene could not decide between these 2 possibilities and we therefore searched for a ring closure for the diaminocarboxylic acid which could decide between a 1,2 and 1,3 diamine. This was accomplished by recourse to the formation of a derivative of the diaminocarboxylic acid with phenanthrenequinone.²⁰ While it is well known that many 1,2 diamines will condense with phenanthrenequinone, there is no evidence that 1,3 diamines form a ring structure with this reagent. The diaminocarboxylic acid when treated with phenanthrenequinone yielded a condensation product melting at 202–204° which crystallized

boxylic acid was a 1,2 diamine and that biotin therefore contained a 5-membered urea ring eliminated two of the five structures which we have been discussing, namely, those containing the 6-membered urea ring—that is, structures V and VI. The diamino-carboxylic acid Ia, Ib or VII could form a phenanthrenequinone derivative.

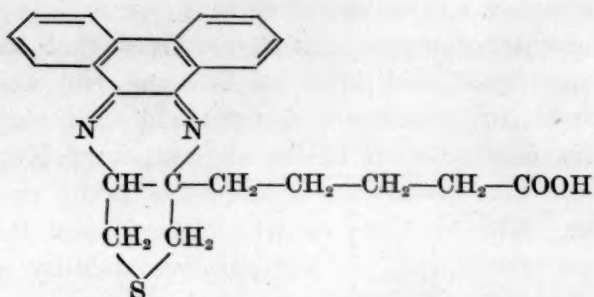
As indicated earlier, the behavior of the phenanthrenequinone derivative of the diaminocarboxylic acid aroused the suspicion that we might have obtained the quinoxaline rather than the dihydroquinoxaline derivative from the reaction of the phenanthrenequinone with the diaminocarboxylic acid, *e.g.*, structure Ia could form a dihydroquinoxaline (formula VIII) but would not be expected to yield the dehydrogenated derivative. On the other hand, the two remaining structures, Ib and VII, can give the dehydrogenated derivative since both carbon atoms bearing the amino groups carry hydrogen atoms. For example, structure Ib can yield a quinoxaline as shown in formula IX.

In order to settle definitely whether or not the derivative obtained from the diaminocarboxylic acid was the dihydroquinoxaline or the more fully aromatic quinoxaline, we asked Dr. Hugh H. Darby at the College of Physicians and Surgeons, Columbia

¹⁹ I. Fankuchen, *Jour. Am. Chem. Soc.*, 64: 1742, 1942.

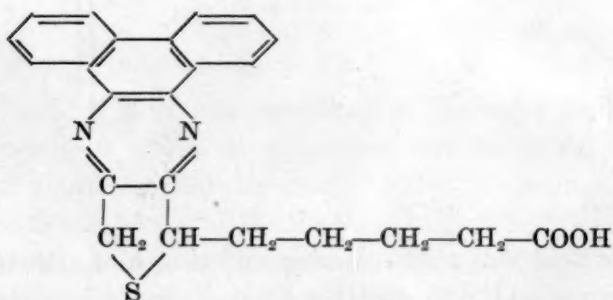
²⁰ K. Hofmann, G. W. Kilmer, D. B. Melville, V. du Vigneaud and H. H. Darby, *Jour. Biol. Chem.*, 145: 503, 1942.

University, to examine the ultraviolet absorption spectrum of the compound and compare it with the spectra of the dihydrodibenzoquinoxaline and dibenzoquinoxaline derivatives of 3,4-diaminotetrahydrothiophene, which we had synthesized (formulas X and XI).



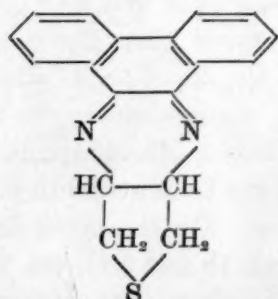
Formula VIII

One would expect a great difference in absorption spectra between these two forms, for one of them, that is the quinoxaline, is more fully aromatic. As we expected, the absorption spectra of these compounds were distinctly different. It was hoped that the absorption curve of the condensation product of the



Formula IX

diaminocarboxylic acid from biotin with phenanthrenequinone would show the characteristics of one or the other of these curves. We found, in fact, that the absorption curve of the derivative from biotin was almost identical in form with that of the oxidized, or quinoxaline, derivative from the 3,4-diaminotetra-

Dihydrodibenzoquinoxaline derivative
Formula X

hydrothiophene, and bore little resemblance to the curve of the dihydroquinoxaline derivative. This is a very strong indication that the derivative formed from phenanthrenequinone and the diaminocarboxylic acid from biotin is a dibenzoquinoxaline, and not a dibenzodihydroquinoxaline, derivative. Thus strong evi-

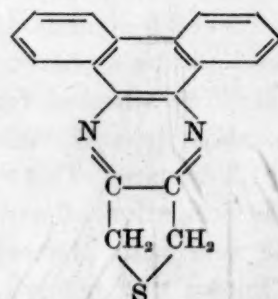
dence was afforded against structure Ia, which left structures Ib and VII still under consideration.

The evidence to decide between these has been obtained in two different ways, one by direct proof and another an indirect approach, beautifully confirming each other and pointing without equivocation to the formula Ib. One was in collaboration with the Merck group I mentioned, and the other by our own group.

The indirect proof of the sulfur ring system has resulted from a collaborative investigation with the group from the Merck Research Laboratory. Dr. Mozingo of their laboratory discovered a very ingenious reaction for removing sulfur from organic sulfides with Raney nickel, in which the sulfur was replaced with hydrogen. They found, for example, that treatment of benzoyl methionine yielded benzoyl-aminobutyric acid, and that the phenyl ureido derivative of methionine gave the corresponding derivative of aminobutyric acid. Still other compounds were studied and it was thought that it might be useful in removing sulfur from biotin and replacing it with hydrogen.

Dr. Donald Wolf applied this reaction in my laboratory to biotin methyl ester and obtained a product containing the same number of carbon atoms and two added hydrogens and no sulfur.²¹ This definitely established the cyclic nature of the sulfide group. This "desthiobiotin" was hydrolyzed to a diamino acid. The desthiodiamino acid derivable from structure Ib possesses only one C-methyl group as indicated in formula XII, whereas that derivable from structure VII (formula XIII) possesses two. A Kuhn-Roth C-methyl analysis showed the presence of one. This result was in favor of structure Ib.

More positive characterization was established by oxidative cleavage. It can be seen that oxidation of the diamine should yield pimelic acid, the 7-carbon

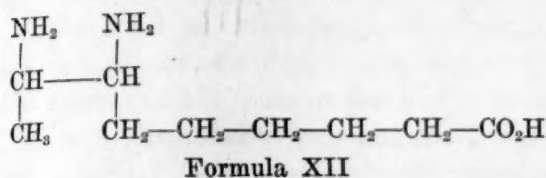
Dibenzoquinoxaline derivative
Formula XI

dibasic acid, if Ib were correct, but α -methyl adipic acid should be formed if VII were correct. Alkaline periodate oxidation yielded pimelic acid, which indicated that the diamino acid was the diamino pellar-

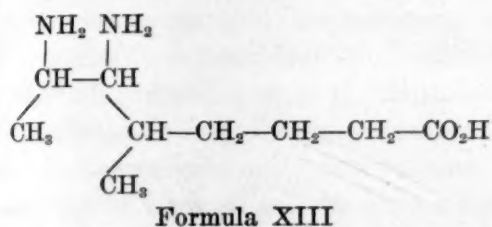
²¹ V. du Vigneaud, D. B. Melville, K. Folkers, D. E. Wolf, R. Mozingo, J. C. Keresztesy and S. A. Harris, *Jour. Biol. Chem.* (in press).

gonic acid. The pimelic acid was identified as such and as its di-p-bromphenacyl ester by comparison with authentic samples of each.

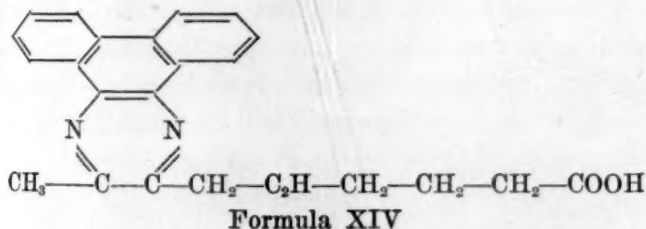
While this work was underway Dr. Folkers and Dr. Harris synthesized the diamino pelargonic acid in the Merck Laboratory. The difficulty we faced, of course, was of comparing the desthiobiotin diamino-carboxylic acid prepared from optically active biotin with the racemic synthetic compound, a difficulty increased by the fact that partial racemization had



apparently occurred in the Mozingo reaction. It occurred to us that these difficulties could be circumvented by preparing the quinoxaline derivatives of both the compound derived from biotin and the syn-



thetic product. As you can see from formula XIV the quinoxaline derivative should possess no asymmetric carbons.

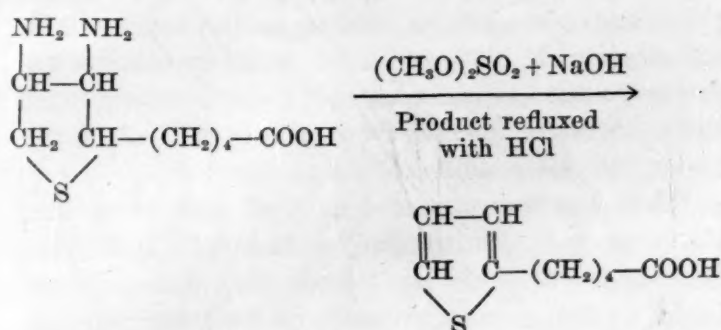


In this way a comparison could readily be made. Consequently Dr. Harris in the Merck Laboratory prepared the quinoxaline derivative of the synthetic compound. Dr. Melville prepared the quinoxaline derivative of the desthiobiotin diamino-carboxylic acid derived from biotin and found that both melted at 186-187° and also that a mixture of the two derivatives showed no depression of the melting point. Thus the desthiobiotin diamino-carboxylic acid was identified as ζ,η -diamino-pelargonic acid.

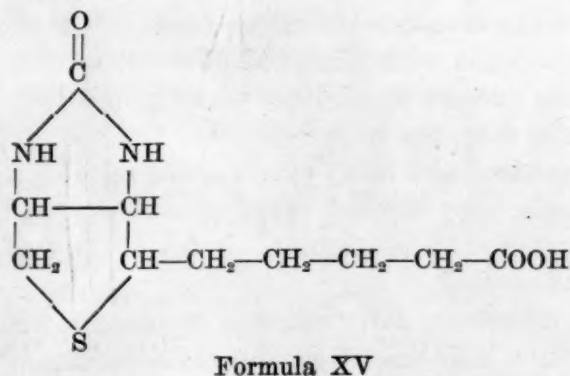
While this collaborative work was in progress we were also continuing in our laboratory another line of attack we had had underway to obtain direct evidence of the nature of the sulfur ring by keeping the sulfur intact. We felt if our formula Ib were correct we ought to be able to obtain a thiophene derivative from it by degradation, and this we could meet by

synthesis. We were convinced that we should be able to obtain δ -(α -thienyl)-valeric acid from the diamino-carboxylic acid. We were so convinced that while the degradation work was progressing we synthesized the compound to have it available for comparison. Dr. Moyer of our laboratory collaborated with Dr. Melville and myself on this phase of our work.

Various attempts were made to bring about the desired decomposition. This was finally accomplished through the decomposition of the methylated diamino-carboxylic acid as shown in the following equation.²²



The compound obtained by this degradation was compared with the sample of synthetic δ -(α -thienyl)-valeric acid. The synthetic compound was prepared by the condensation of thiophene and glutaric anhydride to give a keto acid which was reduced by a Clemmenson reduction to the thienyl valeric acid. This reaction is analogous to that used by Fieser in the synthesis of thienyl butyric acid in which succinic anhydride was condensed with thiophene. The position of the side chain in our synthetic compound was proved by oxidation of the keto acid to α -thiophenic acid. The synthetic δ -(α -thienyl)-valeric acid was found to be identical with the isolated compound, and thus by this step the five-membered sulfur ring with a valeric acid side chain attached to the carbon alpha to the sulfur was directly demonstrated. On the basis of this and the other data I have presented in this lecture we feel justified in concluding that the structure of biotin is that represented by formula Ib, as shown here in full in formula XV.



²² D. B. Melville, A. W. Moyer, K. Hofmann and V. du Vigneaud, *Jour. Biol. Chem.* (in press).

THE MYCOFLORA OF BERMUDA

By Dr. FRED J. SEAVER

NEW YORK BOTANICAL GARDEN

THE Bermuda Islands, because of their nature and location, have always been of extreme interest to botanists as well as vacationists. Since the islands were built up in mid-ocean, and apparently never connected with any other existing body of land, all the land plants originally found there must have come from the outside world through natural agencies, for the islands were uninhabited up to the time of their colonization in 1609, and since many species are endemic it must be concluded that these have originated there through modification of pre-existent forms under extreme conditions of isolation.

When the writer made his first visit more than thirty years ago, in company with Dr. N. L. Britton, to study the flora of these islands, they were a "closed book" so far as our knowledge of the fungi was concerned. Up to that time few American mycologists had touched their shores, and they apparently paid little attention to the fungi so that scarcely more than a score of species were known. In fact, the islands were thought to be barren of this type of growth. However, as a result of our first two-week visit enough species were collected and identified to increase the total number to 122, which number has grown to nearly 400, exclusive of lichens, of which there are 89 species known.

The only other American mycologist who has devoted any considerable attention to the study of Bermuda fungi is Professor H. H. Whetzel, who spent a year there, 1921 to 1922, adding greatly to our knowledge of parasitic fungi, especially the rusts and smuts, and accompanied the writer on his second visit during the winter of 1926. All this work was conducted in collaboration with the local pathologists.

Two return visits were made by the writer, one during the autumn of 1938, and the other covering about the same season in 1940. While these were technically vacation visits, and of short duration, all the time was spent in the investigation of the fungi of the islands in collaboration with Mr. J. M. Waterston, then and up to the present time official plant pathologist. As a result of these two trips twenty-one new species have been described and many others added to the flora of the islands, some of which are of unusual interest from the standpoint of distribution. A few of these will be briefly discussed.

One interesting form collected on our first visit in 1912 was a little scarlet cup-fungus occurring on the dead foliage of native cedar. This was identified as *Sarcoscypha minuscula*, a species which had been described on dead foliage of cedar from Portugal

just one year before we collected it in Bermuda, but nothing seems to have been known of the species in Europe outside of the original collection. While this is one of the more inconspicuous forms, it has been collected by the writer on each of his visits, as well as independently by Professor Whetzel, so that it may be said to be fairly common in those islands. So unique was the species that it was made the type of a new genus in our treatment of the cup-fungi of North America. Nothing more was heard of it until the species was reported from the Yosemite National Park, California, on incense cedar in 1941. This seemed so unusual that in our skepticism we wrote for a specimen of the California fungus. To our surprise the species was absolutely identical with that obtained in Bermuda. So, up to the present time this fungus is known from only three localities in the world: Portugal, Bermuda and the Yosemite National Park, California. It is possible that it is widely distributed and has merely escaped observation, but having been noted so many times in Bermuda by different collectors, this explanation scarcely seems plausible.

One more illustration of European species in Bermuda will be drawn from our pet group, the cup-fungi. Going back again to 1912, on that occasion another cup-fungus was collected which is larger and more conspicuous than the preceding, forming dark purple cups one half to three quarters of an inch in diameter, which by reason of their color contrast strongly with the white sandy soil on which they grow. This species, *Lamprospora Planchonis*, like the preceding, was originally described from Europe with one later collection from North Africa. It may be collected any place throughout the Bermuda Islands. Although known from Europe, and by far the commonest cup-fungus to be found in Bermuda, it has never been reported or seen from the mainland of North America, although the writer has searched for it in similar locations here. From the above it might be assumed that Bermuda had at some time had a land connection with Europe, but let us not be too hasty in our conclusions, for often strictly American species have also been found to be abundant in those islands.

Two illustrations of the latter might be cited. In 1926, in company with Professor Whetzel, an interesting fungus, *Poronia leporina*, was found on rabbit pellicles in one of the smaller islands in Hamilton Harbor. This species was originally described from material collected in Missouri in 1889, and at the time it was found in Bermuda was known from only three collections in America over a period of fifty-three

years. Yet this species was found to be abundant on one of the Bermuda Islands. Unfortunately, we have been unable to revisit this island, and at the present time it has been taken over as an American military base so that we can not check on its reoccurrence.

A second species, rare in America but abundant in Bermuda, is one described as new by the writer more than thirty years ago as *Ophionectria cylindrothecia* from a single specimen collected on cornstalks in Ohio at a much earlier but unknown date. Nothing more was seen or heard of this species until it was found among material on palm stems sent by Professor Whetzel in 1921 from Bermuda for determination. On our later visits to Bermuda it was again collected and found to be exceedingly common and abundant on the native endemic palmetto palm. This species then is known from no other place in the world except from one original collection in Ohio and abundant material obtained in Bermuda at different times and by different collectors. Just why should certain species, exceedingly rare in Europe or America, be abundant in this isolated spot? No explanation can be offered at the present time.

Of more than a score of species described as new to science from material collected on our last two visits, one only will be mentioned here, a subterranean puffball, *Scleroderma bermudensis*, described by Pro-

fessor W. C. Coker, of North Carolina. Remains of this fungus were frequently noted on the sands along the shore and at first taken to be those of an earth-star. It was some time before it was discovered that it was the outer covering of a puffball which during its early stages was entirely concealed in the sand. At maturity the outer covering splits into several rays which bend outward in such a manner as to raise the spore mass as "by its bootstraps" out of the sand where the spores are easily and quickly dispersed by the wind, leaving the remains looking like pieces of dried leather. It is difficult to locate one before the outer covering begins to rupture, at which time this peculiar organism first becomes evident as a crack in the sand. This seems to be another endemic species.

All scientific exploration in these islands, which has become a naval base, has been suspended "for the duration." But there will always be a Bermuda, and it is hoped it may escape the ravages of war, and when the conflict is over it may remain the quiet, restful place so greatly beloved by such men as Woodrow Wilson, Mark Twain (Samuel Clemens) and many other outstanding Americans. At that time we hope we may resume our explorations and researches in that obscure but fascinating field, the mycoflora of Bermuda.

FRED J. SEAVER

THE NEW YORK BOTANICAL GARDEN

SCIENTIFIC EVENTS

DEATHS AND MEMORIALS

DR. CHARLES NELSON HASKINS, Chandler professor of mathematics at Dartmouth College, died on November 14 at the age of sixty-eight years.

DR. LUTHER CROUSE PETER, professor emeritus of ophthalmology of the Graduate School of Medicine of the University of Pennsylvania, died on November 12. He was seventy-three years old.

GEORGE BURR UPTON, professor of automotive engineering at Cornell University, a member of the faculty for thirty-seven years, died on October 2 at the age of sixty years.

DR. ROBERT LINTON, of Los Angeles, consulting mining and industrial engineer, died on November 12 at the age of seventy-two years.

THE New York City Board of Health on November 10 adopted a resolution in memory of the late Dr. S. S. Goldwater for "Raising to new and high levels the standards of medical care." Dr. Goldwater, who had been a commissioner of the City Board of Health, died on October 22.

THE NATIONAL REGISTRY OF RARE CHEMICALS

The National Registry of Rare Chemicals, Armour

Research Foundation, Thirty-third, Federal and Dearborn Streets, Chicago, receives requests for sources of certain chemicals at a rate of approximately two hundred and fifty per month.

Dr. Martin H. Heeren, director of the registry, sends a list of chemicals for which no source is known to the registry. If any reader has one or more in his laboratory, he is urged to communicate with the registry. Even small amounts are important, inasmuch as all requested chemicals are to be used for experimental purposes only.

1. 2,4,6,2',4',6' Hexachloradiazooamino benzene
2. Quinone-bis-beta naphthylimine
3. Porphyrindien
4. 5-Amino-Nicotinic Acid
5. Diethyl Oleyl Amid Phosphate
6. Hexamethylene di iso cyanae
7. Fused Titanium rod 99 per cent. pure
8. CaSi_2
9. Lichenin
10. Pepsinogen
11. 1,8 Dihydroanthraquinone
12. Calcium Sulfaguayacolate
13. Ergotamine Tartrate
14. 2,3,5-triiodophenoxy acetic acid
15. 2,3,5-trichlorophenoxy acetic acid

16. 2,4-dichlorobenzoic acid
17. 2,3,5-trichlorobenzoic acid
18. 2-chloro, 3-nitro-benzoic acid
19. 2,4, diiodophenoxyacetic acid
20. 2,3, dichlorobenzoic acid

THE TRAINING OF WORKERS FOR THE WAR INDUSTRIES

OVER 1,700 war industry workers in New Haven and Fairfield Counties, Connecticut, are enrolled in the Engineering, Science and Management War Training Program for the term that began in the first week of November, according to Forrest Hughes, assistant professor of engineering drawing at Yale University, who is the representative of the university for the organization.

Under the general direction of Yale University and the U. S. Office of Education, training has already been given to 6,300 men and women since the program was begun in 1940 to overcome production bottlenecks. Nearly 3,500 in New Haven and Waterbury have been instructed by the New Haven Y. M. C. A. Junior College, while the Bridgeport Engineering Institute has trained 2,800 workers in Bridgeport and Stamford.

Six new courses are included in the 30 courses offered in New Haven, and three of the 19 courses in Bridgeport will be given for the first time this year. Instruction will be continued in Waterbury and Stamford, while a special course in production planning will be inaugurated at Greenwich and a new school unit will be organized at Meriden in the near future.

Students in these courses are industrial employees who wish to supplement their practical experience on the job with college-level theoretical training to equip themselves for more responsible positions in war industry. About 15 per cent. are women, and this proportion is increasing as more and more women are employed in production. They are found mostly in the courses dealing with inspection, drafting and supervision.

Two of the new courses in New Haven, inspection of aircraft woods and aircraft tool design, were organized at the request of two Connecticut firms manufacturing gliders. Another course, dealing with the surface treatment of metals, will bring the participants in contact with experts on lacquers and oxidizing processes. Those studying materials procurement and control will be taught the procedures and techniques of priorities. Mathematics for industrial electricians will be given as a background course, and a series of classes on the means of maintaining quality standards in mass production with "green" men will also be held. The new courses in Bridgeport will cover the subjects of fuels and their economical use, industrial electricity and fundamentals of radio (advanced).

At the request of the Government and under the auspices of the Engineering, Science and Management War Training Program, there will be given at the University of Illinois a short course which will be repeated as many times as necessary on the techniques and applications of x-ray testing methods, including radiography, microradiography and x-ray diffraction. This is given for the benefit of war industries and Government laboratories which have had to develop x-ray methods in the present emergency, in many cases with technical employees who have not had specialized training. The course as now planned will last for one week, full time. No charge will be made by the University of Illinois to those who attend, since it is being given under Government auspices. Any one who is actually engaged in x-ray testing or who is about to begin this work for any industry or laboratory is qualified and welcome. Application for admission to one of these short-course sessions should be made at once to Professor G. L. Clark, 315 Noyes Chemical Laboratory, University of Illinois, Urbana. It is hoped to organize the first courses early in December. Each session is limited to ten persons.

THE PUBLIC HEALTH RESEARCH INSTITUTE OF THE CITY OF NEW YORK

THE first anniversary of the first public health research institute of any municipality was celebrated on November 1 with the approval by Mayor La Guardia of the first annual report to the Board of Directors of the Public Health Research Institute of the City of New York, Inc., a non-profit scientific research institution. The contract, which was signed on July 1 after the Legislature had passed a bill authorizing cities to enter such agreements, provides for the payment by the city to the institute of \$100,000 annually for a period of ten years, during which it will carry on fundamental research in medicine, biology, physiology, nutrition, public health and other problems of vital interest. The report covers the activities of the institute from July 1 to June 30, 1942, during which period it had carried on research for the city under a temporary contract. It was made public on November 1 by David M. Heyman, president of the board of directors, who is also president of the New York Foundation and the only lay member of the New York City Board of Health. In addition to Mr. Heyman, the board of directors of the new institute includes the Mayor, the Comptroller and the Commissioner of Health as representatives of the city; David Rockefeller as vice-president and David Morse, attorney, as secretary (both now in the Army), and Edwin F. Chinlund, president of the Postal Telegraph, Inc., as treasurer. Accompanying Mr. Heyman's report was a report by Dr. Thomas M. Rivers, director of the Rockefeller Hospital, now commander in the Medical

Reserve Corps, U. S. Navy, who is chairman of the Scientific Council of the institute.

The work of the institute will be carried out by two main divisions. Dr. O. A. Bessey, formerly of the department of pathology of the Harvard Medical School, has been appointed head of the Division of Nutrition and Physiology, and Dr. L. A. Julianelle, of the Washington University Medical School, St. Louis, has been named head of the Division of Infectious Diseases. Dr. Bessey has been appointed director of the institute to replace Dr. Ralph Muckenfuss, who has been called to take charge of the laboratories of the American Expeditionary Forces.

Members of the Scientific Council are Professor Eugene L. Opie, of Cornell University Medical College; Professor Henry C. Sherman, of Columbia University; Dr. George Baehr, of New York, now a colonel in the Army Medical Reserve Corps and chief medical officer of the Office of Civilian Defense, and Professor Michael Heidelberger, of the College of Physicians and Surgeons, Columbia University.

PSYCHOLOGY AND THE WAR

PSYCHOLOGY is becoming increasingly important in the furtherance of our country's war effort. Many will remember that psychology won its spurs as an applied science in the last war. To-day, psychologists are to be found not only in the military service, but in many of the government bureaus.

In the Army, psychologists are serving as classification officers, as personnel technicians and as personnel consultants. The personnel consultant, who is always a commissioned officer, is a man trained in psychology who has demonstrated that he is officer material by passing through an officers' training camp. After receiving his commission, he is eligible for an eight-weeks training course for personnel consultants conducted at the Adjutant General's School, Fort Washington, Md. The personnel officer cooperates with medical and regular officers in the disposition and placement of men. His duties include recommendations for assignment of personnel; supervision of the administration, scoring and interpretation of psychological tests; advice concerning psychological problems involving low-grade men, trouble-makers and others; aid in the selection of men for special duties. The personnel technician performs duties much like those of the personnel consultant, though perhaps not so supervisory. Both may serve as classification officers. Personnel officers are aided by enlisted men who have had some psychological training.

In the Army Air Forces at least 200 enlisted men and 50 officers have been conducting interviews, administering mental, motor and temperamental efficiency tests to aviation cadets. Much of this work is

still experimental. It is under the general direction of Lieutenant-Colonel John C. Flanagan. Psychologists in the Bureau of Aeronautics are engaged in the selection of pilots, bombardiers and navigators. This work, which is carried on in cooperation with the flight surgeon, is under the direction of Lieutenant-Commander John G. Jenkins.

Government agencies such as the U. S. Office of Education, the U. S. Office of Public Health and the U. S. Employment Service all employ psychologists trained in child psychology, mental hygiene, vocational counselling and aptitude analysis. In the U. S. Civil Service, psychologists are engaged also in studies of morale, the interpretation of foreign broadcasts, the conduct of public opinion polls and other activities. Others are at work upon confidential experimental projects concerned with sensory and motor function, and various forms of behavior about which more should be known for effective utilization by the armed forces.

Because of the real need for trained psychologists, Columbia University, in its 1943 Summer Session, is planning a series of courses designed to prepare men and women with adequate undergraduate background for psychological work in the armed forces or in the government agencies. The emphasis in these courses is upon the practical and is applied with a view toward making the services of trained people immediately available.

HENRY E. GARRETT

COLUMBIA UNIVERSITY

THE YERKES LABORATORIES OF PRIMATE BIOLOGY

THE name of the Yale Laboratories of Primate Biology, which up to now has been a department of the Yale School of Medicine, has been changed to the Yerkes Laboratories of Primate Biology in honor of Dr. Robert M. Yerkes, professor of psychobiology, founder and director emeritus of the laboratories.

An announcement made jointly by Yale and Harvard Universities, following a meeting of the newly formed Corporation of the Yerkes Laboratories, also states that the laboratories in Orange Park, Florida, will be conducted by Yale and Harvard Universities with the financial assistance of the Rockefeller Foundation, the Carnegie Corporation and the Samuel S. Fels Fund.

Dr. Yerkes, who continues as professor of psychobiology, has been succeeded in the directorship of the laboratories by Karl S. Lashley, research professor of neuropsychology at Harvard. Henry W. Nissen, associate professor of psychobiology at Yale, continues in the position of assistant director.

The Corporation of the Yerkes Laboratories of Pri-

mate Biology is composed of James B. Conant, president of Harvard University; William B. Claflin, Jr., treasurer of Harvard University; Henry L. Shattuck, fellow of Harvard College; Charles Seymour, president of Yale University; Thomas W. Farnam, formerly associate treasurer and comptroller, and Carl A. Lohmann, secretary, both of Yale University.

Responsibility for research and educational activities will be in the hands of Dr. Lashley and a board of

scientific directors whose membership includes Dean Francis G. Blake, Yale School of Medicine; Leonard Carmichael, president of Tufts College; George W. Corner, director, department of embryology, Carnegie Institution of Washington; Derek E. Denny-Brown, professor of neurology at Harvard; Frederick L. Hisaw, professor of zoology at Harvard; William H. Taliaferro, professor of parasitology at the University of Chicago, and Professor Yerkes.

SCIENTIFIC NOTES AND NEWS

DR. CHARLES-EDWARD AMORY WINSLOW, Anna R. Lauder professor of public health at Yale University, was awarded the Sedgwick Memorial Medal in recognition of distinguished service in public health by the American Public Health Association at the seventy-first annual meeting of the association, held at St. Louis in October.

FIVE Townsend Harris Medals of the Associate Alumni of the College of the City of New York were presented at the annual dinner on November 14. Among the recipients were Dr. William J. Crozier, '12, professor of general physiology at Harvard University; Dr. Selig Hecht, '13, professor of biophysics at Columbia University; and Dr. Alvan L. Barach, '17, assistant professor of clinical medicine at the College of Physicians and Surgeons, Columbia University. The medals were awarded in recognition of "postgraduate achievement."

Chemical and Engineering News reports that E. G. Bailey, vice-president of the Babcock and Wilcox Co., New York, was presented with the first Percy Nicholls Award "for notable scientific or industrial achievement in the field of solid fuels" on September 30 at the banquet of the joint Fuels Conference of the Coal Division of the American Institute of Mechanical Engineers and Fuels Division of the American Society of Mechanical Engineers.

MEMBERS of the American Chemical Society who have been proposed by the local sections for nomination for president-elect are: Thomas A. Boyd, head of the fuel department at General Motors Research Laboratories; Carl Shipp Marvel, professor at the University of Illinois; Thomas Midgley, Jr., vice-president of the Ethyl Gasoline Corporation (now the Ethyl Corporation); Linus Carl Pauling, director of the Gates and Crellin laboratories of the California Institute of Technology; W. T. Read, dean of the School of Chemistry, Rutgers University; Ernest H. Volwiler, vice-president in charge of research and development at the Abbott Laboratories; Hobart H. Willard, professor at the University of Michigan, and Robert R. Williams, chemical director at the Bell Telephone Laboratories.

DR. MARY CAMPBELL BLISS, Margaret C. Ferguson professor of botany at Wellesley College, after serving for forty years, has retired with the title of professor emeritus.

MAJOR GENERAL ROBERT U. PATTERSON, U. S. Army, retired, formerly surgeon general, has been appointed dean of the University of Maryland School of Medicine and College of Physicians and Surgeons, and superintendent of the University Hospital in Baltimore. He succeeds Dr. Hamilton Boyd Wylie, Baltimore, who has been acting dean of the school since the retirement in 1939 of Dr. James M. H. Rowland, Baltimore.

DR. ARTHUR D. HOLMES, research chemist, director of research for the E. L. Patch Company, and Mrs. Arthur D. Holmes, formerly professor of nutrition at the University of Illinois, have been appointed to professorships on the faculty of the Massachusetts State College at Amherst.

ROBERT D. POTTER, science editor of *The American Weekly*, has become instructor in general science at New York University to aid the wartime replacement of teaching personnel now in military and naval service. Mr. Potter will continue his science writing for *The American Weekly*. Losses in the department of general science at New York University include Dr. C. C. Clark, department chairman and now First Lieutenant in the Air Corps, Lieutenant Commander Lawrence Cockaday and Lieutenant T. J. Hanwick, both stationed at Annapolis.

DR. A. C. IVY, professor of physiology at the Medical School of Northwestern University, Chicago, who is now on leave of absence from the university, has been appointed scientific director of the new Naval Medical Research Institute at Bethesda, Md., which will be concerned with the physical and mental condition of aviators, submarine crewmen and marines. The institute was placed in commission on October 27, with ceremonies at which Rear Admiral Ross T. McIntire, surgeon general of the Navy, and Rear Admiral Harold W. Smith, chief of the Division of Research, took part.

DR. WALTER H. EDDY, professor emeritus of physiological chemistry at Columbia University, has been appointed chairman of the department of nutrition and related sciences at the New York Institute of Dietetics.

DR. THURMAN B. RICE, health education consultant to the State Board of Health, Indianapolis, has been appointed acting state health commissioner. Dr. John W. Ferree, Indianapolis, has been granted leave of absence as state health commissioner to serve as lieutenant commander in the medical corps of the U. S. Navy.

Nature reports that the Lord President of the Council has appointed Sir Lawrence Bragg, Professor J. E. Lennard-Jones, Dr. A. McCance and Sir Raymond Streat to be members of the Advisory Council to the Committee of the Privy Council for Scientific and Industrial Research. Dr. G. M. B. Dobson and S. K. Thornley retired from the council on completion of their terms of office on September 30.

ANHEUSER-BUSCH, INC., has established a research unit devoted to the study of the genetics of yeast in the Henry Shaw School of Botany of Washington University, St. Louis. Dr. Carl C. Lindegren has been appointed research associate, Gertrude Lindegren, research fellow, and Grace Schaffel, research assistant.

THE Hawley Products Company, St. Charles, Ill., manufacturers of molded cellulose and allied plastic products, has founded an industrial fellowship in Mellon Institute, Pittsburgh, for the purpose of conducting an investigational program of importance to our armed forces. Dr. J. C. Williams, an alumnus of Oberlin and of the Iowa State College, a specialist in cellulose chemistry and plastics technology, has been appointed to the incumbency of this fellowship. He will be assisted by Peter Shanta, a chemical engineer from the University of Pittsburgh.

PROFESSOR W. L. ENGELS, of the department of zoology of the University of North Carolina, has joined the Army as a private; Professor I. C. Kitchen has enlisted in the Navy as lieutenant (junior grade), and Dr. D. E. Copeland is in the Army as second lieutenant. Dr. Maurice Whittinghill, recently at Bennington College, has been added to the department as associate professor, and Dr. Claude A. Villee, Jr., of the University of California, as instructor; Dr. W. J. Bowen is continuing as assistant professor *ad interim*.

PROFESSOR ALLEN C. TESTER and Professor Joseph J. Runner, both of the department of geology of the State University of Iowa, and Dr. G. C. Knowlton, of the department of physiology, have leave of absence. Professor Tester will begin service with the rank of

captain in the corps of engineers. Much of his work will be in the field of petroleum development. Professor Runner will serve in the U. S. Geological Survey as a senior geologist, devoting his time to a study of copper deposits. Active duty in the Army Air Corps with the rank of first lieutenant is the assignment of Dr. Knowlton.

Nature states that Professor A. V. Hill has heard directly from Professor J. K. Parnas, who was until 1939 professor of medical chemistry in the University of Lwów, that he had succeeded in escaping to the U.S.S.R. and is alive and well.

JOSEPH L. WEINER, deputy director of the Office of Civilian Supply of the War Production Board, will speak on December 11 at the annual meeting of the American Standards Association to be held at the Hotel Astor, New York. Mr. Weiner is also chairman of the Committee of the Government on Concentration of Production in Industry.

PROFESSOR RICHARD H. SHRYOCK, professor of American history at the University of Pennsylvania and lecturer on medical history at the School of Medicine, will deliver a lecture on "Factors Affecting Medical Research in the United States, 1800-1900" at a joint meeting of the Institute of Medicine of Chicago and the Society of Medical History of Chicago at the Palmer House on the evening of November 27.

THE Charles Sumner Bacon Lectures for 1942-1943 of the College of Medicine of the University of Illinois, Chicago, will be delivered on December 2 and 3 by Dr. Edward A. Schumann, formerly professor of obstetrics at the School of Medicine of the University of Pennsylvania.

DR. J. D. BERNAL, professor of physics at Birkbeck College, University of London, delivered at the Royal Institution the first Sir William Bragg Memorial Lecture of the Chemical Society, London, on November 19.

PROFESSOR P. A. BUXTON, director of the department of entomology of the London School of Hygiene and Tropical Medicine, gave on October 30 the first Bacot Memorial Lecture of the Lister Institute of Preventive Medicine.

THE William James lectureship at Harvard University is held this year by Dr. Edward L. Thorndike, professor emeritus of Teachers College, Columbia University. Dr. Thorndike is conducting a seminar during the first half year on "The Applications of Psychological Methods to the Social Sciences," and giving a series of lectures on "Human Nature and Human Institutions," as follows: Oct. 8, "The Original Nature of Man—The Genes of the Mind"; Oct. 15, "Modification by the Environment—Learning";

Oct. 22, "Human Relations"; Oct. 29, "The Psychology of Language"; Nov. 5, "The Psychology of Language" (continued)—"The Origin of Language"; Nov. 12, "The Psychology of Government"—"Rulers and Ruled"; Nov. 19, "The Psychology of Government" (continued)—"Laws and the Law"; Dec. 3, "The Psychology of Punishment"; Dec. 10, "The Psychology of Welfare"—"The Welfare of Individuals"; Dec. 17, "The Psychology of Welfare" (continued)—"The Welfare of Communities." The lectures are open to the public.

THE *Journal* of the American Medical Association reports that the Washington State Department of Health and the U. S. Public Health Service cooperated in the establishment of an industrial hygiene division on October 1. The new division will be housed in the same office building as the State Department of Health.

The *Harvard Alumni Bulletin* states that the latest reports show that four hundred members of the faculty of Harvard University have either left or are on full- or part-time leave for war service. They represent twenty per cent. of the teaching staff. At the Harvard Medical School alone, 180 faculty members have left, many of them to serve in base hospitals overseas from Northern Ireland to the central Army hospital in Australia. Faculty members in many

other departments have been granted leaves of absence either to serve with the armed forces or to engage as civilians in special war work in Washington. Others have been permitted to give full time to research projects financed by the Federal Government and carried on in laboratories at Harvard and elsewhere.

THE *Journal* of the American Medical Association reports that the U. S. Army headquarters for the European theater of operations has announced that the American Red Cross-Harvard University Hospital in southern England has been taken over by the Army and will be the central laboratory for U. S. armed forces in Britain. This hospital was established in 1940 and operated jointly by the American Red Cross, Harvard University and the British Ministry of Health for the study of wartime epidemics. Its twenty-two buildings were all fabricated in the United States, from which the sixty-six thousand pieces of fabricated building material were shipped to England to be erected by British workmen. The director of the hospital was Dr. John E. Gordon, professor of preventive medicine and epidemiology at Harvard University Medical School. The staff comprised ten doctors, sixty-two nurses, six technicians and eight administrative members. The hospital will be turned over to the British Ministry of Health at the end of the war.

DISCUSSION

THE PROBABILITY OF OBTAINING POTENTIALLY DANGEROUS POOLS OF HUMAN SERUM OR PLASMA

THE mixture of plasmas or serums containing antagonistic isoagglutinins results in their inactivation.^{1,2,3} The reaction takes place in a quantitative manner.³ For this reason the practice of pooling serums or plasmas of unknown isoagglutinin content has gained wide popularity. Such pools usually consist of eight to sixteen individual serums or plasmas. Since the groups of the individual components of the pools are not customarily determined, it would seem possible that pools containing disproportionate numbers of serums or plasmas of one type might occur, so that the phenomenon of inactivation might not take place. Such a possibility possesses more than a theoretical danger, since Polayes and Squillace⁴ have reported a near-fatal reaction following the transfusion of pooled plasma. The pooled plasma was later found to be capable of agglutinating the red

blood cells of the recipient. In this paper we shall attempt to demonstrate the mathematical probability of obtaining potentially dangerous pools of human serum or plasma.

In order to simplify the calculation, three assumptions were made: (1) each donor contributes equally to the pool; (2) each sample has the same titer of isoagglutinins; (3) the presence of an excessive preponderance of one group in a pool renders such a pool potentially dangerous for transfusion, *e.g.*, 12 or more samples of Group A or O in a pool of 16; 6 or more samples of Group A or O in a pool of 8; 3 or more samples of Group A or O in a pool of 4.

In this investigation we have used Snyder's⁵ data (based upon 20,000 random samples from the U. S. population) regarding the relative incidence of the four main blood groups: Group O, 45 per cent.; Group A, 41 per cent.; Group B, 10 per cent., and Group AB, 4 per cent.

In order to determine the probability that a certain number of any particular group (O, A, B or AB) of serum or plasma would occur by random sampling of the U. S. population in pools of sixteen, eight and

¹ S. O. Levinson and A. Cronheim, *Jour. Am. Med. Asn.*, 114: 2097, 1940.

² R. Jakobowicz and L. M. Bryce, *Med. Jour. Australia*, 1: 318, 1941.

³ H. A. Davis, *Surgery*, 10: 592, 1941.

⁴ S. H. Polayes and J. A. Squillace, *Jour. Am. Med. Asn.*, 118: 1050, 1942.

⁵ L. H. Snyder, "Blood Grouping in Relation to Clinical and Legal Medicine," Williams and Wilkins Company, Baltimore, 1929.

four sample pools, the binomial expansion was used. Thus the expression $(P_1 + P_2)^n$ may be expanded into the general term:

$$\frac{n!}{a_1! \times a_2!} \times P_1^{a_1} \times P_2^{a_2} = P$$

Where:

P is the probability of obtaining a pool of a_1 samples of one blood group and a_2 samples of any of the other groups when n samples are taken to make the pool and P_1 is the probability of obtaining that group and P_2 is the probability of obtaining any other groups. In the calculations reported here, P_1 took values of 0.45 for group O; 0.41 for group A; 0.10 for group B; and 0.04 for group AB. Corresponding values for P_2 were taken as 0.55 for A, B, AB; 0.59 for O, B, AB; 0.90 for O, A, AB; and 0.96 for A, B, O.

In Table 1 is illustrated the probability of obtaining an excess of any group in pools of 16, 8 or 4 serums or plasmas by random sampling of the U. S. population. As might be expected, the smaller the pool, the greater is the probability of obtaining a preponderant number of serums or plasmas of one group. Moreover, Groups O and A tend to be present with greater frequency in such pools. Defining "potentially dangerous" pools as those containing more than 12 samples of O, A or B bloods in 16, more than six in pools of eight, and more than three in pools of four, then the probability of obtaining potentially

TABLE 1

PROBABILITY (AND APPROXIMATE ODDS) OF OBTAINMENT IN POOLS OF SIXTEEN SERUMS OR PLASMAS

Group	16 times	15 times or more	14 times or more	13 times or more	12 times or more
O	0.000003 (1:330,000)	0.000058 (1:20,000)	0.00048 (1:2,000)	0.00328 (1:300)	0.0143 (1:70)
A	0.0000006 (1:1,700,000)	0.000015 (1:66,000)	0.000173 (1:5,800)	0.00123 (1:800)	0.0062 (1:160)
B	1×10^{-16}	1×10^{-14}	8×10^{-13}	4×10^{-11}	1×10^{-9}
AB	4×10^{-23}	2×10^{-20}	3×10^{-18}	3×10^{-16}	3×10^{-14}

IN POOLS OF FOUR SERUMS OR PLASMAS

Group	8 times	7 times or more	6 times or more
O	0.0017 (1:600)	0.0181 (1:55)	0.0884 (1:12)
A	0.008 (1:1250)	0.01 (1:100)	0.0563 (1:18)
B	1×10^{-8}	7×10^{-7}	2×10^{-5}
AB	6×10^{-12}	1×10^{-9}	1×10^{-7}

IN POOLS OF FOUR SERUMS OR PLASMAS

Group	4 times or more	3 times or more
O	0.04 (1:25)	0.25 (1:4)
A	0.03 (1:33)	0.19 (1:5)
B	0.0001 (1:10,000)	0.0037 (1:270)
AB	0.000002 (1:500,000)	0.0002 (1:5,000)

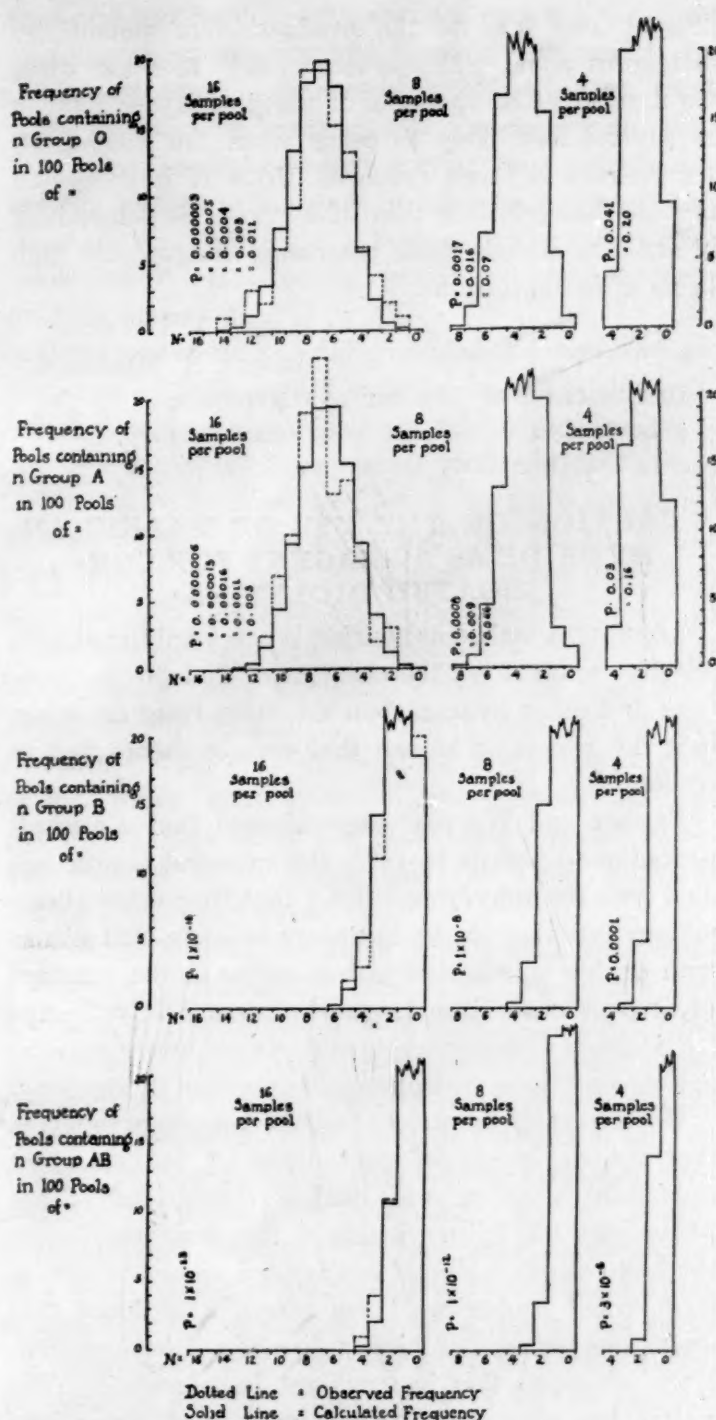


FIG. 1. Frequency distribution of 100 pools of human serum or plasma of 16, 8 and 4 components.

dangerous pools is the sum of the probabilities of obtaining predominantly O, A or B pools. For pools of sixteen, the probability is 0.02 (i.e., odds of 1:50); for pools of eight, 0.14 (odds of 1:7); and for pools of four, 0.44 (odds of 1:2.3). The frequency distribution of groups in 100 pools of 16, 8 and 4 components is shown in Fig. 1. Also shown are the results obtained by actual sampling of marked balls made up to represent the blood group population. It will be noted that the observed frequency follows closely the calculated frequency in the 100 pools containing 16 components.

Certain tentative conclusions may be drawn from these results. The wide-spread practice of pooling serum or plasma without knowledge of the groups

present may lead to the production of potentially dangerous pools. This is more likely to occur when the number of components of the pool is small (such as 4) and less likely to occur when the number of components is large (such as 16). It is suggested that the isoagglutinin titer of all pools be determined in order to exclude those possessing dangerously high titers of isoagglutinins.

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A CAUTION ON THE USE OF MALEIC ANHYDRIDE AS A REAGENT FOR CONJUGATED DIOLEFINS

ALTHOUGH maleic anhydride is commonly used as a selective reagent for the conjugated diolefins in gasolines and other hydrocarbon mixtures from cracking, it is not generally known that certain dienes fail to respond.

Farmer and Warren¹ early showed that 4-methylpentadiene-1,3 fails to form the expected simple adduct with the anhydride. Since that time other observations reported in the literature indicate that dienes with doubly substituted carbon atoms in the terminal (1,4) positions of a conjugated system $RRC = C - C = CRR$ either give polymeric adducts or, under antioxidation conditions, no appreciable reaction of any kind.

More recently the writer and his coworkers reported that the *cis* isomer of pentadiene-1,3 fails to show significant reaction with maleic anhydride.² Since pentadiene-1,3 (piperylene) is the first member in the homologous series of conjugated dienes to exhibit geometrical isomerism, there seems little doubt that analogous isomers of higher dienes will behave similarly, although this has not yet been proved. The writer has also observed in the case of piperylene that the *cis* isomer is much more prominent in mixtures from high temperature processes.

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PYRIDOXIN AND COACERVATES IN PLANT CELLS

PYRIDOXIN may enter into the formation of characteristic aggregates in the vacuoles of senescent or poorly nourished cells which we have recently studied. Free-hand sections of the stems of stunted mustard plants grown without zinc under rigidly controlled conditions show, in the vacuoles of the cells, globular

aggregates which have the characteristics of auto-complex "coacervates." A similar phenomenon has been described whereby the phenolic compounds originally distributed at random in the water phase of the vacuolar solution may be condensed into globular aggregates surrounded by lipoids.¹

Pyridoxin-indophenol may be demonstrated by the Scudi reaction² when free-hand sections of tissues are immersed in an alkaline phosphate or, preferably, veronal buffer, in which 2-6 dichloro quinone chlorimide is suspended. Indophenol first forms where pyridoxin exists, within the coacervates; indophenol, being fat soluble, is then absorbed by the lipid coating the coacervate which it stains blue. The reaction does not occur in a borate buffer where the phenolic group of pyridoxin is known to be masked by the formation of a complex.

We have found *per contra* that in the post-meristematic or the perivascular cells in the roots of mustard or of snapdragon plants grown in a nutrient solution containing zinc and other necessary elements pyridoxin is randomly diffused in the vascular solution. It appears to become "coacervated" in the older cells of plants which remain stunted. A healthy condition is probably dependent upon the presence of pyridoxin in the vacuole. Coacervates may therefore inactivate an important constituent of the cell system.

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X-RAY EVIDENCE FOR A THIRD POLYMORPHIC FORM OF SODIUM STEARATE

THE x-ray work of Thiessen and Stauff gave evidence that there are two distinct crystallographic forms of sodium stearate¹ called by them the α and β forms. The authors have discovered a third form² which may be called the γ form, in conformity with the notation of Thiessen and Stauff.

The new γ form was detected by noting that it had a unique long spacing. The several spacings assumed to be $d_{(001)}$, are as follows:

Form	Spacing
α	51.8Å
β	46.6Å
γ	44.6Å

¹ Howard S. Reed and Jean Dufrenoy. *Am. Jour. Bot.*, 29: 544-551, 1942.

² J. V. Scudi, H. F. Koonen and J. C. Kuesztesy, *Proc. Soc. Exp. Biol. and Med.*, 43: 118, 1940; J. V. Scudi, *Jour. Biol. Chem.*, 139: 707, 1941; O. D. Bird, J. M. Vandenbelt and A. D. Emmett, *Jour. Biol. Chem.*, 142: 317, 1942; J. V. Scudi, R. P. Birks and D. B. Hood, *Jour. Biol. Chem.*, 142: 323, 1942.

¹ *Zeit. Physik. Chemie (A)*, 176: 397, 1936.

² A. de Bretteville, Jr., Thesis under Dr. J. W. McBain, "X-ray Diffraction Study of Oriented Soaps," Stanford University, 1942.

¹ Farmer and Warren, *Jour. Chem. Soc.*, 3221, 1931.

² Robey, Morrell and Wiese, *Jour. Am. Chem. Soc.*, 63: 627, 1941.

The γ form is produced when sodium stearate is formed by the reaction between stearic acid (Eastman catalogue number 402) and sodium alcoholate, followed by drying the precipitate at 105° C.

The authors wish to thank R. D. Vold for preparing some of the soap samples used in this investigation.

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OCCURRENCES OF "RED WATER" NEAR SAN DIEGO¹

SINCE 1917 the Scripps Institution of Oceanography has given considerable attention to the phenomenon of "red water" and to the conditions of its occurrence. Although the information available concerning it is not much greater in detail in 1942 than it was at the time of previous reports,² certain aspects of the conditions appear to be growing more distinct.

1942 was characterized by two periods of "red water," although neither was so conspicuous as in one or two former years. The responsible organisms (dinoflagellates) differed at the two periods in 1942 in contrast to the fact that in the other years only one organism attained "red water" prominence in the year in and near La Jolla Bay. *Prorocentrum micans* Ehr. contributed the color in May, but *Goniaulax polyedra* Stein was causative in September. The duration of noticeable discoloration of the sea in May was about one week, in September about three weeks. The largest abundance recorded in May was 500,000 cells per liter, but probably streaks and patches of more than a million per liter were present. In September the numbers in routine catches at the Scripps Institution pier yielded a maximum of 1,000,000 cells per liter, but special catches showed abundances up to about 2,000,000 cells per liter.

In both May and September the conspicuous populations appeared to drift into the bay from the west. In some other years the invasion was from the northwest. However, in all years the evidence available clearly indicates origins outside of the local area. In 1924 the discoloration caused by *Prorocentrum* was first discovered by the institution boat at about ten miles directly off shore and it was not for several days

that the appearance was distinct in La Jolla Bay. Differences in direction and speed of approach to La Jolla, considered in connection with the fact that there is no recognizable "nursery area" in the region, indicate rather strongly that growth of these populations to "red water" prominence is dependent upon conditions affecting particular masses of water rather than upon conditions affecting particular localized geographic areas.

Perhaps the most striking evidence concerning occurrences of "red water" acquired by the Scripps Institution in twenty-five years is that indicating zonation of conspicuous abundances. Most of this has come to hand since the institution began intensive operations at sea in 1938. From these and from certain earlier observations off shore, it appears certain that high abundance in Southern California does not occur as much as twenty-five miles from shore (possibly not more than fifteen) and that it does not reach to a depth of more than thirty meters (except in very rare instances). By way of contrast, the planktonic diatoms, which usually thrive under conditions apparently favorable to the planktonic dinoflagellates, have shown large populations far from shore, a hundred miles and more.

Here, of course, we have introduced a nice complexity of problems for marine hydrographers and chemists, etc., no less than for marine biologists. How can we account for such definite zonation with water boundaries of one and not the other of two groups closely associated? Still more difficult, how can we account for the fact that within these zones the "red water" organisms may show little prominence for years and then "suddenly" become conspicuous almost over night? Larger and smaller movements of water masses have a very definite place in the results, as do air conditions also, and there must be a long series of chemical and biological influences to run parallel with these when enormous development of numbers occurs. Until we know more about a number of these things our explanations of occurrences of "red water" must remain rather hazy except for some interesting generalities.

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SCIENTIFIC BOOKS

GROWTH AND FORM

Growth and Form. By D'ARCY WENTWORTH THOMPSON. Pp. 1+1116. Cambridge University Press. New edition, 1942. \$12.50.

¹ Contributions from the Scripps Institution of Oceanography, New Series, No. 180.

² W. E. Allen, SCIENCE, 78: 12-13, 1933; SCIENCE, 88: 55-56, 1938.

THIS book, as the author writes in a "Prefatory Note," is a war effort. "I write this book in wartime, and its revision has employed me during another war. It gave me solace and occupation, when service was debarred me by my years. Few are left of the friends who helped me write it, but I do not forget the debt I owe them all." The general character of the work

is shown by the subject-matter of the chapters: Introduction; Magnitude; The rate of growth; On the internal form and structure of the cell; The forms of cells; A note on adsorption; The forms of tissues, or cell aggregates; On concretions, spicules, and spicular skeletons; A parenthetic note on geodesics; The equiangular spiral; Spiral shells of the Foraminifera; The shapes of horns and of teeth or tusks, with a note on torsion; On leaf arrangement, or phyllotaxis; On the shapes of eggs, and of certain other hollow structures; On form and mechanical efficiency; On the theory of transformations, or the comparison of related forms; Epilogue.

There is indeed a continuity of thought in all this strange series of topics, but it comes from mathematics and not from biology. A book of this character and size would be thought to have a profound influence upon biological thinking, but that is not so. An inspection of a series of works upon theoretical biology shows, indeed, only a few references to it, and only rarely is it mentioned at all. How can a work which has cost so much effort have so little effect upon the subject with which it deals, particularly when it is remembered the charm with which it is written? Let us see what statements we can find that will throw any light upon this question.

In his "Epilogue" we find this statement: "For the same reason, with no formal and elaborate conclusion do I bring it to a close. . . . My task is finished if I have been able to shew that certain morphological aspects of morphology, to which as yet the morphologist gives little heed, is interwoven with his problems, complementary to his descriptive task, and helpful, nay essential, to his proper study and comprehension of Growth and Form. . . . And while I have thought to shew the naturalist how a few mathematical concepts and dynamical principles may help and guide him, I have tried to shew the mathematician a field for his labor—a field which few have entered and no man has explored."

There are two men whom he has in mind here—the naturalist and the mathematician, and of the two it is undoubtedly the latter of whom he is thinking the most. Here is biology, with its many problems, and here is a tool, mathematics, that can solve them. Let us present the case and see what will come of it. And so he takes up one problem after the other and shows us what the application of mathematics does for its solution. But driven as he is with the belief in the possibilities of the mathematician as an explainer of the problems of the biologist, one wonders after all just how convinced he is of the value of this transfer

of interest. One encounters every now and then traces of doubt—rarely any firm and unequivocal statements of belief. Thus in the "Introductory" chapter we find these statements: "It is but the slightest adumbration of a dynamical morphology that we can hope to have until the physicist and mathematician shall have made these problems of ours their own. . . . How far even then mathematics will suffice to describe and physics to explain the fabric of the body no man can foresee. It may be that all the laws of energy, and all the properties of matter, and all the chemistry of all the colloids are as powerless to explain the body as they are impotent to comprehend the soul," and then, instead of roundly asserting that this is not so, he mildly adds: "For my part, I think it is not so." He is well aware of the difficulty which the appeal to mathematics makes, for he says: "The introduction of mathematical concepts into natural science has seemed to many men no mere stumbling block, but a very parting of the ways." And in another place he is led to make a statement which raises doubts regarding his own position, for he says: "One does not come by studying living things for a lifetime to suppose that physics and chemistry can account for them all."

There are many other doubts that assail one's mind. One wonders most of all why the entire subject of cytogenetics is left untreated. Surely the significance of all the modern work on this subject must be appreciated, and yet there is no mention of genes and little of chromosomes. Undoubtedly these are of the utmost significance in the determination of growth and form in development. Again, in considering the relation between molecules and the living system there is no reference to supramolecular units, a conception of the greatest significance. Indeed, while the title of the work is "Growth and Form," the act of becoming is only incidentally treated. This the author recognizes, for he states: "My sole purpose is to correlate with mathematical statement and physical law certain of the simple outward phenomena of organic growth and structure, or form, while all the while regarding the fabric of the organism, *ex hypothesi*, as a material and mechanical configuration."

How far it is possible to go, considering only the ill-defined forces in the completed structure, it is difficult to say, but surely it can be asserted with little fear of contradiction that in the present work the author leaves us in the end pretty much where we were in the beginning. But even at that one can not blame the author, for neither in the beginning nor at the end, when all the arguments are made, does he make any definite promises. For us he has outlined a series of

problems in biology, to which he has suggested mathematical solutions and, having done that, he dismisses the whole matter in the hope that some eminent

mathematician will be inspired to take advantage of the opportunity to make of biology a true science.

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SPECIAL ARTICLES

PATHWAY OF INVASION IN A CYNOMOLGUS MONKEY AFTER ORAL APPLICATION OF POLIOMYELITIS VIRUS¹

DESPITE a great deal of work on both human and experimental material, the portal or portals of entry of poliomyelitis through the body surfaces has not as yet been precisely determined. Present evidence shows that the olfactory system is not as a rule primarily implicated, and that invasion probably occurs in most cases through the alimentary tract, but it is not yet known whether the upper portion—the mouth and pharynx—or the lower portion—the stomach and intestines—is the more vulnerable to penetration by virus.

In studying this problem we have encountered certain technical difficulties which have apparently prevented others also from obtaining a clear answer. Among these may be mentioned the difficulty of confining the application of virus in the experimental animal to a particular region of the alimentary tract; and the difficulty, in cases of fully developed infection, of determining the portal of entry from the distribution of virus or of lesions in the central nervous system. The latter procedure appears to be better adapted to the exclusion of a given portal than to its positive determination, because, once virus has become implanted and the animal has developed typical symptoms of the disease its spread is remarkably rapid and extensive, even before paralysis has occurred.

It has become clear to us that the experimental animal must be sacrificed at the earliest possible moment of manifest infection or even before this, in order to obtain plain evidence of the primary localization. However, the adoption of routine and systematic examination of the peripheral nervous ganglia—suggested by McClure's recent work²—has proved to be helpful, a method the value and significance of which has been surprisingly late in gaining recognition. These ganglia contain the nerve cells whose axons supply the mucous surfaces through which the virus presumably first gains access to the interior of the body and it is highly probable that they are the primary site of multiplication of this strictly neuronotropic virus. Since most of the ganglia (with the exception

of those of the vagus) supply a fairly limited portion of the mucous surfaces, the distribution of lesions or of virus in them should afford valuable clues to the portal of entry, provided as we have stated that the examination is made early in the disease. Particularly significant ganglia are the Gasserian, the geniculate and the petrosal, which supply the mucous membranes of the mouth and nasopharynx, and the celiac, which supplies the stomach and intestine. The sympathetic and spinal ganglia also have some localizing value, but the vagus ganglia (nodose and jugular) have such wide-spread connections in the entire alimentary and respiratory tracts as to give but little localizing information.

Using cynomolgus monkeys and Sabin's *Per* strain of poliomyelitis virus, we have applied virus to various parts of the alimentary tract and have killed animals shortly after the onset of fever or, in some cases, without any signs of infection. Tissues of the central and peripheral nervous system have been systematically examined for lesions, including the olfactory bulbs, brain stem and spinal cord; the ganglia of the V, VII (geniculate), IX, X cranial nerves; the sympathetic ganglia of the ganglionated cord at all levels; the spinal ganglia at all levels, and the celiac plexus. The results of the study as a whole will be reported later, but at this time we wish to present the data in one monkey as being of some special interest.

Cynomolgus 9. Capsules, covered with a digestible fat, containing dried virus amounting to about one third of a cynomolgus cord, were inserted into the esophagus on April 5, 1941, in such a manner as to avoid contamination of the mouth. On May 20 and again on September 15, 1941, after zinc sulfate olfactory blockade, the tongue was gently swabbed with a minute amount of 15 per cent. virus suspension. On January 22, 1942, a high enema of 5 cc of 20 per cent. virus suspension was administered. No symptoms and no fever occurred after any of these treatments. The olfactory mucosa was again treated with zinc sulfate on March 14, 1942, and on March 25 and on each of the 3 following days, the mouth was sprayed from an atomizer with 5 cc of a 10 per cent. suspension supernate. On March 30, 5 days after the first spraying, fever, slight weakness of the arms and mild head tremors were noted at 5 P.M. (none of these had been present that morning). It is highly probable that this animal would have become paralyzed. It was sacrificed about 15 minutes later, following our routine

¹ From the Department of Pediatrics, Stanford University School of Medicine, San Francisco, Calif. Sponsored by the National Foundation for Infantile Paralysis, Inc.

² G. Y. McClure, *SCIENCE*, 94: 307, 1941.

procedure of etherization, exsanguination, perfusion with physiological saline solution and 10 per cent. formalin. The nervous tissues were stained with galloeyanin and eosin according to Einarson's method.

Serial sections were made of the olfactory bulbs and peripheral ganglia. Sections of the brain stem were taken at intervals of 0.6 mm and of the spinal cord from upper, middle and lower portions of the cervical, thoracic and lumbar regions (3-4 successive sections, 20 micra thick from each). Lesions³ in the ganglia consisted of small cell infiltrations, chromatolysis, neuronal necrosis and neuronophagia; in the brain stem, mainly of perivascular infiltrations with occasional parenchymal infiltration. Heavy lesions were found in both Gasserian ganglia and both nodose (X nerve) ganglia. Moderately severe lesions were found in both petrosal (IX nerve) ganglia, in 3 of 6 cervical sympathetic ganglia and 2 of 10 upper thoracic ganglia. Small lesions, few in number, were found in one geniculate (VII nerve) ganglion; in 1 lumbar sympathetic ganglion and in 2 of 14 thoracic spinal ganglia. In the medulla a few typical parenchymal and perivascular infiltrations, without definite cell necrosis, were found in and near the nucleus of the tractus solitarius but nowhere else. No lesions were found elsewhere in the brain stem, in the olfactory bulbs, the lower thoracic sympathetics, the celiac, the cervical spinal or the lumbar spinal ganglia or in the spinal cord.

With the exception of the few very slight lesions in one lumbar sympathetic ganglion (which may have resulted from inapparent infection from the earlier virus enema), it would appear that the lesions found can all be explained on the basis of nerve-borne infection entering through the mouth, mainly through the fibers of the fifth, ninth and tenth nerves (probably including the gustatory), and to a lesser extent through the sympathetics; and it is evident that infection had just begun to invade the central nervous system from its primary neuronal site of multiplication through the central connections of the IX and X

³ In one control cynomolgus monkey fairly numerous infiltrative lesions were found in several of the peripheral ganglia. This animal had been kept for over 10 months in the same animal room with others that had been freely exposed to virus by enema, etc. The lesions resembled in all respects those found in animals treated with poliomyelitis virus. While it is possible and perhaps probable that this was an instance of "spontaneous" poliomyelitis infection, one must be guarded in concluding that lesions in the ganglia are necessarily due to poliomyelitis and not to some other neurotropic virus. In the present instance the prompt sequence of infection after mouth exposure, to poliomyelitis virus, the localizations of the lesions and the typical early lesions in the medulla are believed to make the diagnosis of poliomyelitis reasonably secure. McClure (personal communication) has also found lesions in the peripheral ganglia in rhesus monkeys not directly treated with poliomyelitic material.

cranial nerves in the nucleus of the tractus solitarius in the medulla. It is interesting to note that although the Gasserian ganglia were heavily involved, their central connections contained no lesions. The dorsal motor nucleus of the X nerve likewise was uninvolved.

The experiment is of special interest because it demonstrates entry of virus through the mouth and pharynx after oral administration, and also the mode of progression of infection from the exterior mucous surfaces to the local peripheral nervous system into the central nervous system. It would appear that the mouth and pharynx are readily vulnerable to penetration by this virus. We are far from wishing to use this experiment or others like it as evidence in exclusion of other possible portals, such as the lower alimentary tract. Indeed, we have some positive evidence that infection can also enter via the latter. However, it may be pertinent to note that the very frequent occurrence of headache, vomiting, nuchal pain and other symptoms in the preparalytic stage of the human disease strongly suggest early involvement of the brainstem, particularly the medulla, which is better accounted for by entry from the oropharyngeal passages than from the more distant intestines. It has too generally been assumed that bulbar paralysis is the sole criterion of primary bulbar poliomyelitis. Primary involvement of the afferent centers is, in our opinion, of at least equal importance and possibly more common. The reason why bulbar motor involvement is frequent after adenotonsillectomy is probably that infection is traumatically introduced into the motor nerves of the pharynx and so conducted directly to their motor nuclei. In the ordinary case, in which deep trauma is not a factor, infection entering through the oropharyngeal membranes would rather invade the afferent nerves into the peripheral ganglia and thence into the sensory bulbar centers, whence it could progress to other centers but without necessarily involving the motor nuclei of the medulla. Experimentally, it has been thoroughly proven that, following olfactory and intracerebral inoculation, poliomyelitic infection can pass freely down through the medulla into the spinal cord and produce spinal paralysis without accompanying bulbar paralysis.

Since poliomyelitis is probably acquired as a rule by the alimentary route, the oropharyngeal mucosa is obviously the first site of contact with virus in contaminated food and drink and on fingers, which children so often put in the mouth. Here the contacts of the virus with the mucous surfaces are immediate and the virus is at its maximum concentration. In the stomach and upper intestine, dilution by secretions and destruction by acid and proteolytic enzymes may to some extent protect the body against infection. In the present experiment the failure of large amounts

of virus to infect the same animal when previously administered by stomach without mouth contamination gives some support to such a concept.

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**A VIRUS OBTAINED FROM A PNEUMONIA
OF CATS AND ITS POSSIBLE RELATION
TO THE CAUSE OF ATYPICAL
PNEUMONIA IN MAN**

A RESPIRATORY tract infection in cats—variously called nasal catarrh, influenza or distemper—has been observed frequently within the past year or so in the Northeastern United States. The main characteristics of the disease are its highly infectious nature, debilitating effects and long course of about a month. Its respiratory nature is recognized by sneezing and coughing, which is accompanied by a mucopurulent discharge from the eyes and nose. The existence of a pneumonia is not determined by the usual clinical examination unless the animal is markedly affected, but an autopsy reveals grayish, densely consolidated areas in the anterior lobes of the lungs.

Suspensions of lungs from cats showing typical clinical symptoms and pneumonia were inoculated intranasally into mice. The mice became sick in the first passage, and those inoculated with two of the strains died in 3 to 5 days. In another attempt to isolate the agent, the mice appeared sick but survived the inoculation. At autopsy all inoculated mice presented a definite pneumonia with more than half the lung substance consolidated. Serial passage reduced the time interval to a point where death occurred in 2 to 3 days following the intranasal inoculation of a 10 per cent. suspension of infected lungs. Similar serial passages from uninoculated mice from the same source were entirely negative.

The agent was easily transferred to eggs, which had been incubated for 5 days, by inoculation into the yolk sac. The embryos died consistently within 2 to 3 days in serial passage, even when relatively large amounts of infectious material were inoculated.

When suspensions of lungs of inoculated mice or of yolk sac membranes of inoculated eggs were given intranasally to normal kittens the typical disease was produced. From these inoculated cats the disease went by contact to normal kittens.

Cultures from the lungs of naturally infected cats and of infected mice showed few bacteria and were frequently negative. All attempts failed to demonstrate a cultivable agent from infected eggs on blood agar plates and on a variety of special media designed for the culture of anaerobes and pleuropneumonia-like forms. These findings suggest that the agent is a virus, yet attempts to pass the agent through Berke-

field filters gave irregular results. The nature of the agent, however, became apparent when sections of the yolk sac membrane stained with Giemsa, or films from lungs of mice or yolk sac membrane treated by Machiavello's method, revealed numerous elementary bodies similar to those of psittacosis.

Centrifugation of infected mouse lungs and yolk sac suspensions at 10,000 r.p.m. for 30 minutes removed much of the infective agent from the supernatants and concentrated it in the sediments. This is added evidence that the observed elementary bodies are the etiological agent.

A number of instances of contact between sick cats and people who subsequently developed atypical pneumonia have been brought to our attention. For example, Dr. Francis G. Blake (personal communication), of Yale University, observed an atypical pneumonia in a rural family in Connecticut which occurred where cats were sick with a pneumonia. Dr. C. W. Barber, of the New York State Veterinary College, noted the reverse, where a child sick with atypical pneumonia played with a kitten that later became sick. It may be of epidemiological interest that the disease in man and in cats is occurring simultaneously.

Complement fixation tests have been made, using antigens of partially purified and concentrated elementary bodies prepared from infected mouse lungs. Sera obtained from cats before infection and again after they had recovered were tested. All the 6 cat sera obtained before infection failed to fix complement when 0.1 cc or less was mixed with 0.1 cc of the antigens. Using the same amount of antigen and testing at the same time, the convalescent cat sera fixed complement when from 0.02 to 0.0025 cc was used. Five sera drawn from man during the acute and convalescent stages of atypical pneumonia were obtained from Miss Catherine Greni and Dr. Norman Moore, Cornell Infirmary, Ithaca, New York, and 7 more similar sera from Dr. Frank Horsfall, of the Hospital of the Rockefeller Institute. Eight of these sera drawn during the acute illness fixed complement; and the convalescent sera from 5 of these cases showed a definite increase in this property, while 3 showed a questionable increase and 4 no increase.

Sera from 9 presumably normal individuals were examined for controls. 0.1 cc of 2 specimens failed to fix complement, while the same amount of 4 others fixed more or less completely; 2 specimens fixed with 0.05 cc and 1 with 0.025 cc. As controls in this test, one serum drawn during the acute stage of the disease fixed in an amount of 0.0125 cc, while the convalescent serum fixed in $\frac{1}{4}$ this amount, or 0.0031 cc. Another serum drawn during the acute stage failed to fix when 0.1 cc was used, whereas 0.0125 cc of the convalescent serum fixed.

SUMMARY

Evidence is produced that a respiratory disease in cats is due to a virus that forms elementary bodies and that this virus is the same as or closely related to the one causing some of the so-called atypical pneumonias in man. Further work is in progress, and a detailed report of the cat disease and of additional complement fixation tests will be made later.

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CAPILLARY EMBOLI AS A LETHAL FACTOR IN BURNS¹

In the course of an extensive series of experiments on shock following thermal trauma in anesthetized cats,² it was noted that a small proportion of the animals succumbed with extreme rapidity within a few minutes of burning. In these cases, respiration stopped suddenly, the heart was slow and irregular and the blood pressure fell precipitately. Artificial respiration usually did not result in recovery, although the heart continued to beat for some time. Hemoconcentration and local fluid loss into the area of the burn were minimal at the time of death. Necropsy in these cats showed pulmonary congestion, subendocardial hemorrhages in the left ventricle and in one instance punctate hemorrhages in the liver and submucous hemorrhages in the duodenum.

Since the sudden death in these experiments could not be explained on the basis of loss of fluid and since the nervous factor had been eliminated experimentally,² the phenomenon was attributed to a toxin. The investigation was carried forward to study the possibility that changes produced by heat in the circulating blood could account for rapid death from burns.

Measurements of subcutaneous temperature in experimental scalds³ show that temperatures of 55 to 65 degrees C. are reached and maintained for several minutes. Citrated cat's blood was heated to 65° C. (149° F.) for one minute; it remained fluid but became dark in color and showed evident hemolysis. Heating of plasma to 56°–65° C. resulted in the formation of a voluminous fine precipitate, presumably fibrinogen. On the other hand, when serum was treated in the same way, no visible change was evident. Fresh coagulable blood heated quickly to 65° C. remained fluid and became incoagulable.

After heating a small amount of citrated cat blood for one minute to 65° C., reinjection intravenously

into the same animal resulted in rapid death. This reaction was similar to the rapid deaths observed following burns. Blood pressure fell markedly and respiration ceased within one to two minutes, but the heart continued to beat for 5 to 10 minutes and artificial respiration was of no avail. As little as 3 to 4 cc of heated blood injected rapidly produced this characteristic fatal outcome. The same phenomenon has been observed repeatedly in cats under nembutal following rapid intravenous injection of 1 to 2 cc of autogenous plasma previously heated to 65° C. for one minute. Intravenous injection of heated serum in much larger amounts had no significant effect. The supernatant of centrifuged heated plasma was also ineffective, indicating that it is the fine precipitate of fibrinogen in heated plasma which possesses the toxic properties.

By very slow intravenous injection of heated plasma, much larger amounts could be introduced without producing rapid death. In one experiment, after injection of 13 cc of heated plasma, blood pressure was low for an hour and respiration became rapid and shallow. The cat survived for four days, during which time it was very weak and unresponsive and made no spontaneous movements. Necropsy showed marked congestion of the lungs, slight renal congestion, a discolored dark gray and softened liver and a gastric ulcer one cm in diameter which had perforated.

The experiments were then extended to study the effect of intravenous injection of heated human plasma and serum into unanesthetized rabbits. Human plasma heated to 56° to 65° C. showed a precipitate similar to that observed in heated cat plasma, while heated human serum was apparently unchanged. Rapid injection through a 22 gauge needle of 2 to 5 cc of heated human plasma into the ear vein of rabbits resulted in rapid arrest of respiration followed by anoxic convulsions with occasional gasping. The heart continued to beat for some time, but artificial respiration was ineffective. All the rabbits injected with heated plasma died within 5 to 10 minutes. The centrifuged precipitate of heated human plasma gave the same reaction, while intravenous injection of as much as 40 cc of the supernatant or of unheated human plasma had no effect. Intraperitoneal injection of heated human plasma was also ineffective. Rapid intravenous injection of heated human serum in amounts up to 40 cc was likewise innocuous to the rabbits.

Necropsy of rabbits which died from injection of heated human plasma or precipitate showed no gross pathology in the internal organs or in the brain other than occasional slight pulmonary congestion. Microscopic examination of the lungs revealed wide-spread and numerous capillary protein emboli.

¹ Aided by a grant from the Graduate School of the University of Minnesota.

² H. Kabat and R. F. Hedin, *Surgery*, 11: 766–776, 1942.

³ H. Pfeiffer, *Virchow's Arch. f. path. Anat.*, 180: 367, 1905.

The precipitate from heated human plasma (presumably fibrinogen) was rendered innocuous simply by homogenizing it in an apparatus capable of breaking up tissue cells. Rapid intravenous injection of 40 cc of the homogenized suspension of the precipitate had no toxic effect on rabbits. This demonstrates conclusively that it is the particle size rather than the chemical constitution of this material which bears the toxic properties.

There is a distinct possibility that capillary emboli may play a role in the constitutional effects of severe burns. Many of the older investigators on pathogenesis of shock and toxemia in burns considered this possibility. Frankel⁴ noted minute capillary thrombi in the liver, spleen and kidneys in burn cases, and

Bardeen⁵ reported that capillary thrombosis in the liver was not infrequent following burns. Billroth⁶ and others supported the embolic theory of the etiology of Curling's ulcer. In experimental burns, Salvioli⁷ found that previous defibrination of the blood rendered dogs more resistant to burns. He showed that warming of the mesentery to 55° C. caused adherence of platelets to the walls of small vessels and formation of minute thrombi. Vaccarezza⁸ observed hypocoagulability of the blood following burns in dogs.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE METHOD FOR RAPID TUBE FEEDING OF RATS¹

OUR attempts to forcibly feed rats have developed a method permitting one person to feed rats rapidly without recourse to expedients such as gags,^{2,3} holding the tongue,⁴ wrapping the animal in towels⁵ or clipping the teeth.⁶ The method is described here in the hope that it will prove of value to others confronted with this problem.

The equipment required is: (1) a syringe of appropriate size; (2) a 7-inch piece of soft rubber catheter tubing (No. 8 Fr.), one end being cut square and the other beveled; (3) an adapter made by soldering a piece of brass tubing (1 cm long, 2 mm o.d.) to the hub of an old hypodermic needle and inserted into the square-cut end of the catheter tube; (4) a ring stand and burette clamp; (5) a piece of heavy string about 30 inches long having a large securely tied loop at each end.

The feeding is done as follows: The syringe is filled with the desired quantity of the liquid food mixture and is securely clamped to the ring stand in a horizontal position by means of the burette clamp. The rat is grasped in the left hand with the thumb and index fingers about the shoulders and the left foreleg held between the index and middle fingers. The right foreleg is supported by the thumb. With the rat in a

vertical position facing the operator, one loop of the string is caught over the upper incisors, the string is passed over the back of the left hand and between the fourth and little fingers and is fastened by winding it several times about the latter. The tension on the string should be sufficient to hold the head of the animal securely against the palm of the hand. The other loop of the string is now caught over the lower incisors, passed down over the back of the thumb, and fastened by winding around the middle and fourth fingers of the left hand. Tension should be sufficient to hold open the animal's mouth, the extent of opening being controlled by pulling apart the thumb and index fingers which respectively increase the tension on the lower and upper jaws of the animal. The rat is thus effectively suspended by the two strings attached to its jaws. Undue pressure can not be exerted on the animal, for, in order to hold it securely, the thumb and index fingers must be held apart.

With the animal in a vertical position and resting its hind feet on the operator's chest, the wetted tube is inserted into the esophagus with a downward rotating motion. Using a soft rubber tube we have not once, in the course of several thousand feedings, inserted the tube into the trachea. When the tube is pushed down sufficiently (depending on the size of the rat), the adapter is firmly attached to the syringe and the contents of the latter are slowly forced through the tube. The tube is withdrawn and the string attached to the lower jaw is unwound from the fingers.

⁴ E. Frankel, *Deutsche med. Wchnschr.*, 15: 22, 1889.

¹ Aided by a grant from the Rockefeller Foundation and administered by Dr. P. E. Smith.

² R. M. Reinecke, H. A. Ball and L. T. Samuels, *Proc. Soc. Exp. Biol. and Med.*, 41: 44, 1939.

³ C. S. Mathews, E. L. Schwabe and F. E. Emery, *Jour. Lab. and Clin. Med.*, 27: 352, 1941.

⁴ A. E. Pugh and A. W. Tandy, *Jour. Lab. and Clin. Med.*, 24: 80, 1938.

⁵ D. J. Ingle, Personal communication.

⁶ R. M. Reinecke and L. T. Samuels, *Endocrinology*, 30: 687, 1942.

⁵ C. R. Bardeen, *Johns Hopkins Hosp. Rep.*, 7: 137, 1898.

⁶ T. Billroth, *Wien med. Wchnschr.*, 17: 705, 1867.

⁷ J. Salvioli, *Virchow's Arch. f. path. Anat.*, 125: 364, 1891.

⁸ R. A. Vaccarezza, *Comp. rend. Soc. de Biol.*, 86: 1114, 1922.

The animal is placed on the table in an upright position and the loop holding the upper teeth flicked off in a single motion. The syringe and tube are rinsed with water and are ready for the next animal.

After short training a single operator can feed 30 to 40 animals per hour. Regurgitation or leakage up the esophagus is never encountered. By the use of this method we have supplied normal and hypophysectomized rats of all ages, beginning at 35 days, with their entire food supply for long periods with excellent results.

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THE USE OF CREOSOTE IN MOUNTING FLEAS AND OTHER ARTHROPODS ON SLIDES

To study the classification of fleas properly, it is necessary that unsclerotized structures and intestinal contents be cleared or dissolved away so as to expose the taxonomically important terminalia. In general, the procedures used to accomplish this end are long and tedious. It is usually considered necessary to treat the material with potassium hydroxide, dehydrate in several changes of alcohol and clear in xylol before mounting in balsam. The technique of C. Fox¹ makes eight treatments necessary before the flea is ready for study, while that published by the writer² in 1940 involves six steps, which is but a slight saving in time and trouble.

In an effort to discover a method of preparation which would dispense with potassium hydroxide, and the necessity for dehydrating and clearing in separate processes, experiments were made with cedar oil, clove oil, beechwood creosote and wood creosote. It was soon discovered that the best of these reagents for this purpose is wood creosote. Creosote not only clears the soft parts and intestinal contents to a satisfactory degree, but also prepares the specimen for mounting in balsam. No other reagent is necessary. The flea may be removed from any degree of alcohol or even from water and placed in creosote for 24 hours. Thereafter it is ready for mounting in balsam. Both the creosote U.S.P. from wood tar and the creosote U.S.P. from beechwood were satisfactory.

The chief advantage to this method of preparing fleas is the convenience of having to use but a single reagent. There are other advantages, however, to the use of creosote instead of KOH. It frequently happens in the use of KOH that important taxonomic characters are distorted, the setae are loosened and lost, and in general much destruction of parts in-

flicted. These things do not happen where creosote is used, and it is the writer's opinion that a much better mount is obtained, since sufficient clearing is accomplished without the violent action of a caustic. A disadvantage to the use of creosote is its slightly irritating effects to the human skin and the objectionable odor, but the writer does not regard these as annoying to a prohibitive degree.

This simple process has proved a boon not only as regards research in the taxonomy of fleas, but also in preparing material for use by large classes in entomology. Thrips, Collembola, mites, immature stages of Diptera, and insect organs, such as honeybee stings, mouthparts, etc., have been prepared quickly and easily by simply dropping the material in creosote and mounting in balsam after 24 hours. Where the integument is rather delicate, as in the case of some Collembola, it is preferable to "cut" the creosote with equal parts of absolute alcohol. The process should not be used, however, where the integument is very delicate or where it is desirable to retain the coloration.

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¹ Carroll Fox, "Insects and Disease of Man," p. 221. Philadelphia, Pa., 1925.

² Irving Fox, "Fleas of Eastern United States," p. 2. Ames, Iowa, 1940.